

# CHARACTERIZING NITROGEN LOSSES TO AIR AND DRAINAGE WATER FROM RED CLOVER MANAGED AS GREEN MANURE OR FORAGE

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By  
Brian Mark Wallace

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## ABSTRACT

The transfer of N from legume green manures (GMr) can satisfy the needs of a successive cash crop, but rotations that have over-wintering legumes also carry an increased risk of off-season (Sep.–June) N losses, especially during spring thaw. Spring-wheat yield among four GMr systems were evaluated with respect to off-season (GMr; Sep.–June) and in-season (wheat; June–Sep.) N<sub>2</sub>O emissions, as well as full-year NO<sub>3</sub><sup>−</sup> leaching and dissolved N<sub>2</sub>O losses during spring-thaw from a tile-drained sandy loam soil in Atlantic Canada over 2 rotations (2011–2013). Four GMr systems (treatments) differed in the timing and season of GMr incorporation and the use of additional N as fertilizer or manure. The majority (66%) of cumulative N<sub>2</sub>O emissions were measured during the off-season because of high N<sub>2</sub>O emissions events during spring thaw. There was no clear effect of GMr system on these emissions, which may have been a result of the pattern and duration of soil freezing and thawing. Spring thaw also coincided with the highest dissolved N<sub>2</sub>O concentrations (100–300 µg N<sub>2</sub>O-N L<sup>−1</sup>) in tile-drained water, which represented potential N<sub>2</sub>O emissions of 21 to 116 g N<sub>2</sub>O-N ha<sup>−1</sup>. Belowground N<sub>2</sub>O concentrations and soil water content measurements during winter provided further evidence of the relationship of N<sub>2</sub>O dissolved in drainage water and N<sub>2</sub>O emissions at the soil surface. Wheat yield among treatments in either year of study were not different, but was 1.5 times greater in Year 2 (2.62 ± 0.27 Mg ha<sup>−1</sup>), than Year 1 (1.05 ± 0.12 Mg ha<sup>−1</sup>). The highest NO<sub>3</sub><sup>−</sup> concentrations in drainage water (Oct.; 13.8 mg NO<sub>3</sub><sup>−</sup>-N L<sup>−1</sup>) were measured from the GMr system with the earliest fall incorporation (i.e., Sep.) and the addition of spring fertilizer when compared to the mean of all other treatments (9.8 mg NO<sub>3</sub><sup>−</sup>-N L<sup>−1</sup>). The use of supplemental N did not translate into additional gains in yield, yet increased in-season N<sub>2</sub>O emissions and greater NO<sub>3</sub><sup>−</sup> leaching. Off-season N losses proved to be a substantial part of the annual N loss budget and dissolved N<sub>2</sub>O in drainage water was identified as an additional pathway for N loss at spring thaw.

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## LIST OF ABBREVIATIONS

|                     |  |
|---------------------|--|
| BNF                 | Biological N <sub>2</sub> fixation   |
| DM                  | Dry matter   |
| DNRA                | Dissimilatory nitrate reduction to ammonium  |
| EF+N <sub>70</sub>  | Early Fall + N fertilizer at 70 kg ha <sup>-1</sup>                                    |
| GHG                 | Greenhouse gases   |
| GMr                 | Green manures  |
| HLF+M <sub>70</sub> | Hayed Late Fall + liquid dairy manure; target N rate of 70 kg ha <sup>-1</sup>         |
| LF                  | Late Fall  |
| N <sub>min</sub>    | Soil inorganic nitrogen (NH <sub>4</sub> <sup>+</sup> + NO <sub>3</sub> <sup>-</sup> ) |
| NUE                 | Nitrogen use efficiency  |
| Q <sub>10</sub>     | Temperature coefficient  |
| RSMN                | Residual soil mineral nitrogen   |
| SNS                 | Soil nitrogen supply   |
| SP                  | Spring   |
| WFPS                | Water filled pore space  |
| ΣFDH                | Sum of Freezing Degree Hours (<0 °C)   |
| ΣTDH                | Sum of Thawing Degree Hours (>0 °C)  |
| ΣDH                 | Sum of Degree Hours (ΣFDH+ ΣTDH)   |

## **1. GENERAL INTRODUCTION**

### **1.1 Introduction**

Although there is little evidence to support the claim that organically produced food has increased nutritional benefits (Magkos et al., 2003; Johansson et al., 2014), the North American consumer is more likely motivated by the “improved nutritional value” when purchasing organic food, while European consumers are focused on the benefits to “environmental stewardship” or animal welfare (Lund, 2006; Lynch, 2009). Including a diversified crop rotation and increasing soil inputs of crop residues and manures can benefit soil and plant health and cumulatively can reduce off-farm inputs of pesticides and soil nutrients. Research that takes more of a systems approach to the interacting and cumulative effects of the social, ecological, and economic goods and services (Lynch et al., 2014) could persuade the North American consumer of other benefits from the organic industry. Despite the limited organic agricultural research in Canada, the demand for organic food continues to grow, which contributes added pressure on organic farmers to increase yields while minimizing environmental degradation.

Organic farming systems in eastern Canada use biennial legumes as green manures (GMr) in forage–grain crop rotations to increase soil fertility and to improve overall soil conditions from the input of organic matter. With these improvements to soil quality, there is also an indication of increased N losses to air and drainage water when legumes are the dominant GMr plant species (Bergstrom and Kirchmann, 2004; Gregorich et al., 2005; Rochette and Janzen, 2005; Korsath, 2008; Jensen et al., 2012). Because biennial GMr accumulate large amounts of N in above and belowground biomass (Hatch et al., 2014), N losses are likely to occur outside of the main growing season before the planting of the intended crop—especially in eastern Canada where edaphic and climate conditions can encourage N loss.

The majority of the soil N cycle is mediated through the microbial community that begins when organic N is transformed to inorganic N, or atmospheric N<sub>2</sub> is symbiotically converted to a

biologically usable form (Robertson and Vitousek, 2009). Biological properties that are coupled to soil physical characteristics regulate O<sub>2</sub> availability and water movement through the soil profile (Blagodatsky and Smith, 2012), which can lead to gaseous and aqueous N losses to the environment. The greatest N loss pathway occurs as NO<sub>3</sub><sup>-</sup> leaching; especially during periods of high precipitation and limited plant uptake when residual soil mineral nitrogen (RSMN) is high following harvest (Rasouli et al., 2014). Nitrous oxide also receives significant attention because 50 to 64% of total N<sub>2</sub>O emissions are from agricultural soils (Janzen, 1998; Burton et al., 2008), as well as N<sub>2</sub>O being associated with processes that lead to ozone destruction (Cicerone, 1989; Mosier et al., 1998; Butterbach-Bahl et al., 2013). The numerous N<sub>2</sub>O production and consumption processes occurring in soil are likely occurring concurrently, but denitrification and nitrification activities are dominant in most arable soils (Firestone and Davidson, 1989; Pennock et al., 2006). Nitrification related processes are more dominant in semi-arid environments (e.g., Ma et al., 2008; Bedard-Haughn et al., 2013), while denitrification produces greater N<sub>2</sub>O in more humid climates (Wagner-Riddle et al., 2008; Burton et al., 2012). There is also a risk of N<sub>2</sub>O losses occurring from drainage tiles (Burton et al., 2012) because N<sub>2</sub>O is highly soluble in water and has a high probability of downward movement in soil water when soil N<sub>2</sub>O concentrations are elevated (Heincke and Kaupenjohann, 1999).

The timing and season of GMr incorporation may be modified in an effort to minimize N losses, but there are factors other than environmental N losses that are considered when cropping patterns are designed. In particular, the ability for a farmer to access a field with heavy machinery could be limited by high precipitation and saturated soil conditions. In an effort to regulate the timing of soil N availability, the season and timing of GMr incorporation can be modified, as well as the use of spring added supplemental N as fertilizer or manure. In eastern Canada, early fall incorporation has a high risk of off-season (i.e., fall to spring) N losses as soil mineralization and nitrification processes will likely not be limited by a lack of soil water or soil temperature. Spring GMr incorporation may allow for better protection against off-season N losses, but the soil N supply (SNS) may not be well synchronized with crop uptake and could potentially reduce cash crop yields. Delaying fall GMr incorporation until Nov. is an alternative cropping practice that has had some success in maintaining an adequate SNS while minimizing off-season N loss (Sanderson and MacLeod, 1994; Carter, 1994; Sanderson et al., 1999; Lahti and Kuikman, 2003). Decomposition is delayed at this time of year due to cooler soil

temperatures, but then proceeds the following spring without having to wait for ideal soil conditions for tillage, thereby reducing the amount of field preparation needed to prepare the seedbed of the succeeding crop. Removing aboveground GMr biomass as hay, or using it as forage with a later application of manure, are other GMr management options. Although GMr decomposition and N loss are highly dependent on annual weather patterns, several GMr management strategies could be used to minimize N loss while maximizing the transfer of N to the cash crop.

The likelihood of N losses due to leaching or gaseous emissions during the off-season is positively related to the soil  $\text{NO}_3^-$  content, but diminishes when a grass or legume crop is planted (i.e., catch crop) after harvest (Bouwman et al., 1993; Askegaard et al., 2011). The increase in the  $\text{NO}_3^-$  pool could either be a result of reduced uptake by the cash crop, or the increase could occur before cash crop planting due to early or rapid GMr decomposition. The focus around GMr tillage and the timing of incorporation is typically based on managing the aboveground biomass, yet much of the accumulated organic N from the fertility building phase of the rotation is located belowground in the rhizosphere (Walley et al., 2007; de Vries and Bardgett, 2012; Arcand et al., 2014). The contribution to soil inorganic N ( $\text{N}_{\text{min}}$ ) from the belowground biomass has been estimated at 30–75% (Evans et al., 2003), but would be accelerated with tillage. Thus, all GMr management strategies, regardless of the seasons and timing of aboveground biomass incorporation, have the potential to increase soil  $\text{NO}_3^-$  availability and the risk of environmental N losses, especially without a growing crop during the off-season.

The overall objective of this study was to evaluate N losses from four GMr rotation systems as it moved from the GMr phase into the cash crop phase of the rotation. A secondary objective was to evaluate how the cash crop acted as an N sink as a result of the four different GMr systems. Having each phase of the rotation present in every year allowed us to examine two distinct years with respect to year to year weather differences. Treatments differed in management by the timing of GMr incorporation, and the use of supplemental N as fertilizer and dairy manure along with the removal of aboveground biomass. Nitrogen losses in this study included off-season soil  $\text{N}_2\text{O}$  emissions from the GMr phase of the rotation and in-season losses from a spring wheat crop. Total  $\text{NO}_3^-$ -N load in drainage water was measured year-round and represented losses from the entire 4-yr crop rotation (Soybean–GMr–GMr–Wheat). In addition to



$\text{NO}_3^-$  leaching,  $\text{N}_2\text{O}$  dissolved in drainage water was assessed through the winter and as soil thawed.

## **1.2 Organization of the Dissertation**

Research presented in this dissertation is organized in manuscript format and presented in Chapters 3 through 5 following this introduction and the literature review in Chapter 2. All research chapters represent work conducted from the spring of 2011 until the spring of 2013 at one research site in Truro, NS, Canada. The site is located within an area that is certified for organic agricultural research and undergoes an annual audit of the agronomic practices used in accordance to Canadian standards for organic agriculture. The experimental site also includes a network of sub-surface drainage tiles where water quality and drainage volume can be evaluated from 12 isolated plots (16 x 75 m). The field experiment was designed as a four year crop rotation where each phase of the rotation was present every year—an aerial image of the site along with a schematic of the plot layout is found in Appendix 1.

The first research chapter (Chapter 3) describes  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions from a 2-yr-old red clover stand during the off-season (i.e., Sep.–June) over two years. Weather data along with soil water and temperature measurements are used as indices of physical properties occurring at the surface and within the soil profile that may have a control on the timing and magnitude of  $\text{N}_2\text{O}$  emissions. I hypothesized that GMr treatments that were incorporated in fall would have greater off-season  $\text{N}_2\text{O}$  emissions when compared to spring incorporated GMr, but delaying fall incorporation until Nov. would reduce these emissions. Because this study is solely focused on the GMr phase of the rotation during the off-season (Sep.–June) there is no use of supplemental N in any of the treatments.

The second research chapter (Chapter 4) investigates the below ground gas concentrations of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  within the 7.5 to 57.5 cm soil profile from GMr plots during the off-season. The content of  $\text{N}_2\text{O}$  dissolved in drainage water due to the four year crop rotation is also examined. Treatments and plots used in this study are identical to those used in Chapter 3. Belowground  $\text{N}_2\text{O}$  concentrations would act as an indicator for potential  $\text{N}_2\text{O}$  losses at the soil surface and dissolved  $\text{N}_2\text{O}$  leaving the site in drainage water was the tested hypothesis.

Chapter 5 outlines growing season (June–Sep.) soil N dynamics as a result of the varied GMr systems and plant N uptake and yield of spring wheat, while measuring in-season CO<sub>2</sub> and N<sub>2</sub>O emissions and full-year NO<sub>3</sub><sup>−</sup> leaching over the entire four year crop rotation. The hypothesis used in this study was that moderate additions of supplemental N, in the form of manure and mineral fertilizer, would improve yields of spring wheat as compared to using GMr alone without increasing N losses to air and drainage water.

A synthesis of the major findings from all three research chapters is discussed in Chapter 6 and evaluates the clover management systems with respect to the seasonal dynamics of N losses through the various pathways discussed above and their ability as a GMr to supply N to the successive crop.

## **2. LITERATURE REVIEW**

### **2.1 Organic Agriculture and GMr**

In contrast to natural ecosystems that have a relatively tight N cycle where peak availability of soil N coincides with maximal plant uptake, agricultural systems are notorious for low N efficiencies (Christensen, 2004). Decades of research have been dedicated to modern solutions that increase N efficiency by improved inorganic fertilizer formulations, improved plant cultivars, and the timing and method of soil inputs of organic N in the form of manures, composts, and plant residues. Although the principles are deeply rooted in the past, there has been a rise in organic agricultural research with the goal of designing a system that better mimics a natural system (Dawson et al., 2008). The ability to supply enough essential plant nutrients to satisfy crop yields without using inorganic fertilizers has not slowed the acceptance of organic agriculture, but designing a system that optimizes plant uptake with soil nutrient availability has proved to be one of the greatest challenges of organic agricultural systems (Berry et al., 2002; Watson et al., 2002).

Increasing N use efficiency (NUE)—defined here as the ratio of plant uptake to N input—in cropping systems is considered to be the best method to decrease environmental N losses (Davidson et al., 2014). In agricultural systems that rely on inorganic fertilizers the NUE is estimated to be around 50% (Conant et al., 2013), but has been increasing recently due to improved crop yields and N application rates that have leveled off in North America (Davidson et al., 2014). In organic systems, strategies to enhance NUE would include a better synchronization of the SNS from legume residues with varied timing of GMr incorporation and the use of supplemental N (Lynch et al., 2012), and use of deep rooted catch crops (Thorup-Kristensen, 2006). Other work being done that is specific to organic research, is focused on the breeding of plant varieties that are selected to be more productive in lower N environments because of more extensive root systems (Dawson et al., 2008) and is a promising strategy for the future. Regardless of the production practices used, reducing the amount of reactive N in the

environment is a critical goal for all agricultural systems (Galloway et al., 2002) so that yields continue to increase without further environmental pollution.

Although the yield gap between conventional and organic agricultural systems can be substantial (Dawson et al., 2008), benefits of organic agricultural practices on a soil's internal N cycle may require a longer time period before a pronounced effect on productivity becomes apparent. In comparison to continuous corn, a diversified crop rotation and greater organic matter inputs have increased yield of the cash crop in some long-term studies (i.e., Drury et al., 2014a); however, short-term changes to physical or chemical soil properties may be difficult to quantify following a shift in management (Nelson et al., 2009; Knight et al., 2010). Some of the key soil properties that benefit from increased inputs of organic matter include: improved soil structure (e.g., Tisdall and Oades, 1982; Thorup-Kristensen et al., 2003), increased soil water content and improvements to infiltration (Franzluebbers, 2002), greater nutrient availability from increased aggregate size and stability (Kong et al., 2007), and less soil degradation (Breland, 1995). Biological properties of soil quality may be more sensitive to changes in management, as compared to chemical or physical soil properties, especially when the distinct change in management comes from the increased input of organic matter. A larger microbial biomass has been associated with agricultural systems that include legumes in the rotation (McDaniel et al., 2014a), which has been shown to improve ecosystem N retention (de Vries and Bardgett, 2012) as well as the resistance to pests and disease in higher plants (Peoples et al., 1995). Crop rotations and the input of GMr residues can also increase net N mineralization (Sanchez et al., 2001), and the ability to decompose recalcitrant residues (McDaniel et al., 2014b). There are numerous benefits to soil properties from using a diversified crop rotation and increasing crop residue inputs, however, the possibility of negative consequences of greater N availability needs to be considered as N loss is positively related to N availability during periods without a growing crop.

The use of legumes grown as GMr is an essential component of soil fertility management plans on organic farms because the high costs or minimal availability of animal manures often restricts its use, leaving few other options for producers in these systems (Knight et al., 2010; Loes et al., 2011). Organic cropping systems in Atlantic Canada are characterized by extended (4–5 yr) rotations that typically include two years of leguminous GMr that are grown as forage, or to build soil fertility (Lynch et al., 2008; Lynch, 2009; Nelson et al., 2009). Red clover

(*Trifolium pratense* L.) is a biennial/perennial species that can be grown as a winter cover crop (Gentry et al., 2013), or maintained for multiple years when biomass is either removed and used as forage (Thilakarathna et al., 2012), plowed under in spring or fall (Thorup-Kristensen and Dresboll, 2010), or cut and left on the surface as a mulch (Hatch et al., 2014; Woodley et al., 2014). The amount of N supplied to a succeeding crop will vary with the species grown, edaphic properties, and the timing and method of termination (Liebman et al., 2012). However, for every Mg of cereal grain produced, the soil needs to supply 20 to 40 kg N ha<sup>-1</sup> to satisfy adequate seed and vegetative production in these crops (Peoples et al., 1995). Estimates of biological N<sub>2</sub> fixation (BNF) by red clover fluctuate widely (Watson et al., 2002), yet on average 25 kg N Mg<sup>-1</sup> of biomass is fixed from the atmosphere in aboveground portions of the plant (Peoples and Baldock, 2001; Carlsson and Huss-Danell, 2003), while 30 to 40 kg N has been estimated when belowground portions of the plant are included (Peoples et al., 2009). Therefore, for systems relying on GMr for N fertility alone, one or two Mg dry matter (DM) ha<sup>-1</sup> of above and belowground GMr biomass is needed for every Mg of grain produced. This estimate works on the assumptions that N inputs from the native soil organic matter and N losses somewhat cancel each other out, and legume BNF is maximized. However, like conventional producers who apply fertilizer in excess of crop demand as an inexpensive insurance policy (Davidson et al., 2014), organic producers need to maximize yields by adding N in excess of crop demand, but the timing of the soil N supply (SNS) can be much more difficult to predict. Determining best management of GMr in organic production systems that produce acceptable yields with minimal N losses require further study (Liu et al., 2010), especially for humid maritime climates in eastern Canada.

## **2.2 Soil Nitrogen Transformations**

Optimum conditions for biological activity and N mineralization occur around 30°C and a soil water content that is 50 to 80% of field capacity, or a water filled pore space (WFPS) of 60% (Bouwman et al., 1993; Voroney, 2007; Whalen and Sampedro, 2010). Soil biological activity can be compared among systems by the increase in production of CO<sub>2</sub> with temperature; commonly described by a  $Q_{10}$  temperature function of around two, over a 15 to 35°C soil temperature range (Voroney, 2007)— $Q_{10}$  is the proportional change in respiration with a temperature increase of 10°C. Although activity in colder soils is much slower, the  $Q_{10}$  value for

soil temperatures close to 0°C were recently reviewed to be 4.8 over a range of cold soil environments (Hamdi et al., 2013). In these conditions, N transformations are still occurring, although different microbial species with unique temperature sensitivities are involved.

Nitrous oxide is of particular interest as a GHG because of its global warming potential, which is 298 times that of CO<sub>2</sub> (molecule for molecule) (Mosier et al., 1998), and its role in the destruction of stratospheric ozone (Cicerone, 1989; Butterbach-Bahl et al., 2013). Nitrous oxide is mainly produced through aerobic and anaerobic denitrification pathways, and as a by-product of nitrification soil processes (Firestone and Davidson, 1989; Davidson et al., 1991). The chemical decomposition of hydroxylamine during autotrophic and heterotrophic nitrification, and chemodenitrification of soil nitrite are likely only small contributions to overall N<sub>2</sub>O production (Butterbach-Bahl et al., 2013). Aerobic processes include nitrifier-denitrification from organisms involved in nitrification as well as coupled nitrification–denitrification by distinct organisms of each process when soils transition to anaerobic status (Wrage et al., 2001; Kool et al., 2011). When soil O<sub>2</sub> becomes limited, denitrification can occur from a diverse group of organisms that use N oxides as alternative electron acceptors during respiration (Burton et al., 2012). Nitrate ammonification, or dissimilatory nitrate reduction to ammonium (DNRA), is yet another process that produces N<sub>2</sub>O in soil (Schmidt et al., 2011).

Availabilities of soil NO<sub>3</sub><sup>−</sup> and C, in addition to aeration, are known to be important factors controlling denitrification (Firestone and Davidson, 1989; Davidson, 1991); however, the ratio of NO<sub>3</sub><sup>−</sup> to C and soil temperature have a strong control on N<sub>2</sub>O:N<sub>2</sub>+N<sub>2</sub>O molar ratio during this process (Beauchamp et al., 1989; Morkved et al., 2006; Gillam et al., 2008; Mitchell et al., 2013). Because of the greater energy potential of NO<sub>3</sub><sup>−</sup> over N<sub>2</sub>O, NO<sub>3</sub><sup>−</sup> is preferentially used as a terminal electron acceptor over N<sub>2</sub>O by denitrifying organisms (Firestone et al., 1979; Cho et al., 1997), which can slow the complete reduction of N<sub>2</sub>O to N<sub>2</sub>. The availability of C influences N<sub>2</sub>O production because of its role as an energy source and electron donor for heterotrophic organisms involved in N<sub>2</sub>O production. For example, at low NO<sub>3</sub><sup>−</sup> levels and increased C availability, the N<sub>2</sub>O:N<sub>2</sub>+N<sub>2</sub>O molar ratio is decreased; resulting in a greater proportion of N<sub>2</sub> being produced during denitrification (Kramer et al., 2006; Mitchell et al., 2013), whereas N<sub>2</sub>O emissions are favored as NO<sub>3</sub><sup>−</sup> and C availability increase (Miller et al., 2008; 2009). Furthermore, increased microbial activity when C availability is high can also decrease soil O<sub>2</sub> availability, creating conditions conducive to denitrification (St. Luce et al., 2011; Burton et al.,

2012). Indeed, the relative composition of  $\text{NO}_3^-$ , labile C, and  $\text{O}_2$  in a soil has a complex control on  $\text{N}_2\text{O}$  production and emissions from soil.

Emissions of  $\text{N}_2\text{O}$  have been positively correlated with soil inorganic N intensities, calculated as the time-weighted sum of  $\text{NO}_2^- + \text{NO}_3^-$  (e.g. Burton et al., 2008; Zebarth et al., 2008; Asgedom et al., 2014) or  $\text{NO}_2^-$  over a defined season (e.g., Maharjan and Venterea, 2013). Inorganic N intensity is then used as an index of the magnitude and duration of inorganic N exposure to microbes involved in  $\text{N}_2\text{O}$  production. Nitrite intensity explained a greater share of the variability in  $\text{N}_2\text{O}$  emissions over  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in a fertilizer source and placement study in Minnesota, USA (Maharjan and Venterea, 2013), as well as a  $^{15}\text{N}$  tracing study that observed  $\text{NO}_2^-$  being the typical precursor to  $\text{N}_2\text{O}$  production in the laboratory (Russow et al., 2009). The lack of routine soil  $\text{NO}_2^-$  measurements because of added cost and the necessity of immediate analysis explains why  $\text{NO}_2^-$  as a substrate for  $\text{N}_2\text{O}$  production is not traditionally expected or discussed discussion with  $\text{N}_2\text{O}$  production in most agricultural studies. However,  $\text{NO}_2^-$  rapidly reacts to form  $\text{N}_2\text{O}$  under a broad range of aerobic conditions (Venterea, 2007) and has a closer proximal position to  $\text{N}_2\text{O}$  in most  $\text{N}_2\text{O}$  producing mechanisms when compared to  $\text{NO}_3^-$  (Venterea and Rolston, 2000) and should be considered when evaluating management practices that reduce soil accumulation of  $\text{NO}_2^-$  may also reduce  $\text{N}_2\text{O}$  emissions (Maharjan and Venterea, 2013).

### **2.3 Timing of GMr Incorporation**

Net mineralization and nitrification in soil at a particular point in time is best described as an index of the competing processes of immobilization and mineralization (Schimel and Bennett, 2004), and ultimately plant uptake and N losses (St. Luce et al., 2011). Mineralization will be dominant over immobilization when added crop residues have a C:N ratio that is less than 20 (Thorup-Kristensen et al., 2003) and will also affect the overall rates of these processes occurring over time. A comparison of crop rotations with and without legumes found that a smaller overall amount of biomass returned to the soil from the rotation with legumes produced similar gross mineralization and nitrification rates over a growing season in a semi-arid environment because of the greater quality (i.e., C:N) of the legume residue (Bedard-Haughn et al., 2013). Crop sequence within a rotation can have significant effects on mineralization rates from year to year,

as available C and N pools are influenced by the preceding crop and will vary with management (Doran et al., 1987), yet these rates are difficult to predict across varying soil conditions and year to year (Phillips, 2008).

In temperate climates, fall GMr incorporation may increase over-winter  $\text{NO}_3^-$  leaching as N immobilization processes are more sensitive to low soil temperatures than nitrification (Hoyle et al., 2006), and mineralization (Andersen and Jensen, 2001; Magid et al., 2001; Lahti and Kuikman, 2003), although results may differ in the presence of a readily available C source (Cookson et al., 2002). Alternatively, spring incorporated GMr eliminates winter fallow and the risk of increased N loss, but establishment of the succeeding crop may be hampered by such factors as prolonged N immobilization (Francis et al., 1992), reduced germination (Lynch, 1980), and increased competition from annual and perennial weeds due to the lack of weed control during the GMr phase of a rotation (Askegaard et al., 2011). Finally, fall GMr incorporation may be more desirable, as spring field preparation may be delayed in heavier soils during years with heavy precipitation or excessive snowmelt because of saturated soil conditions that limit field accessibility with heavy equipment. Soil properties and climatic conditions will influence the timing of GMr incorporation. Having a variety of GMr incorporation options to ensure minimal N losses and a sufficient soil N supply to satisfy cash crop needs is necessary.

Fall and spring incorporation of GMr for spring wheat production in Finland was examined by Lahti and Kuikman (2003), both in the field and laboratory, where treatments included early fall incorporation (Sep. 1), delayed incorporation (Oct. 20), late fall (Dec. 23), and spring (May 3). Greatest wheat grain yields were achieved from late fall incorporation ( $4.03 \text{ Mg ha}^{-1}$ ) as compared to early incorporation in September (lowest yield:  $2.79 \text{ Mg ha}^{-1}$ ). This contradicts the findings of Francis et al. (1995) who did not observe a treatment effect on wheat yield from delayed fall GMr incorporation. However, there was a yield depression when GMr was incorporated in spring attributed to immobilized N early in the growing season (Francis et al., 1995). Understanding how the timing of GMr incorporation influences yields of the succeeding crop, and the associated pathways for N loss, will provide organic producers with options that can be designed for their soil conditions and production systems.



## 2.4 NO<sub>3</sub><sup>-</sup> Leaching

Nitrogen is lost from the root zone as a result of NO<sub>3</sub><sup>-</sup> leaching, which is thereby controlled by N mineralization and nitrification rates, crop uptake, manure or fertilizer additions, precipitation, soil thawing, and the timing of tillage (Dinnes et al., 2002). The loss of inorganic N occurs predominately as NO<sub>3</sub><sup>-</sup> because NH<sub>4</sub><sup>+</sup> is held much more tightly in the soil matrix because of its positive charge and the predominance of negative charged exchange sites in soil (Brady and Weil, 2001). An increasing number of agricultural soils in eastern Canada have been classified as having a high risk for NO<sub>3</sub><sup>-</sup> leaching, especially between growing seasons (Milburn et al., 1997; De Jong et al., 2007; Drury et al., 2007; Lynch, 2009). In areas of potato cultivation on PEI, NO<sub>3</sub><sup>-</sup> concentrations in soil leachates can reach as much as 65 mg L<sup>-1</sup> (Savard et al., 2007), but diminish through the winter (Zebarth et al., 2003). Synchronizing the SNS with crop uptake and the inclusion of cover crops in GMr systems can reduce NO<sub>3</sub><sup>-</sup> leaching (Francis et al., 1992; Drinkwater et al., 1998; Baggs et al., 2000; Stopes et al., 2002; Lahti and Kuikman, 2003; Eriksen et al., 2004; Jabloun et al., 2015). However, microbial mediated N transformations do not solely occur during the period of crop uptake, and could be significant when the timing of GMr tillage occurs outside of the main growing season (Dahlin et al., 2005), especially in fields with sub-surface drainage tiles (Drury et al., 2014b).

The use of animal manures along with a range of organic farming practices were investigated for N leaching during 3 full rotations of a 4-yr crop rotation in Denmark (Askegaard et al., 2011), where the application of conventional animal manure is limited to 70 kg total N ha<sup>-1</sup> yr<sup>-1</sup> in an effort to limit excessive NO<sub>3</sub><sup>-</sup> leaching. A three treatment factorial experiment included the following factors: (1) proportion of grass/clover in rotation, (2) with and without an over-winter catch crop, and (3) with and without animal manure. There was no effect of manure application on N leaching, which implies that moderate manure applications in the spring will likely not lead to reduced NUE or a large residual N pool. The biggest contributor to N leaching was the inclusion of an over-winter cover crop, and more specifically, the frequency and intensity of fall tillage.

Soil inorganic N (N<sub>min</sub>) content from the 0–30 cm soil depth on 228 commercial potato fields over three years was sampled at planting and harvest to relate N content to cropping history and weather in New Brunswick, Canada (Zebarth et al., 2003). Although there was a high

degree of variability among fields and years, low  $\text{NO}_3^-$  contents in spring were related to years that had precipitation amounts greater than normal in the preceding fall and spring and higher  $\text{NO}_3^-$  contents in spring were related to relatively drier conditions. Soil  $\text{N}_{\min}$  in spring was controlled by climatic conditions and the history of the preceding crop. More specifically, low  $\text{NO}_3^-$  contents at the 0–30 cm soil depth were likely a result of leaching and/or denitrification in wet years while high soil  $\text{N}_{\min}$  in spring (e.g., 2 to 124 kg N ha<sup>-1</sup>) was as a result of a preceding red clover or hay crop (Bélanger et al., 2000; Zebarth et al., 2003).

## **2.5 Legume GMr and N<sub>2</sub>O Emissions**

Soil  $\text{NO}_3^-$  levels not only influence N loss through leaching, but are positively correlated with N<sub>2</sub>O emissions as well (Bouwman et al., 1993; Chirinda et al., 2010a; 2010b). Emissions of N<sub>2</sub>O from Canadian agricultural operations are estimated to be 80 Mg N<sub>2</sub>O-N yr<sup>-1</sup>, or 60% of the total anthropogenic related emissions (Janzen, 1998) and are primarily associated with denitrification soil processes in humid soil moisture regimes (Mosier et al., 1998; Burton et al., 2008). During the GMr phase of a rotation, N<sub>2</sub>O production is likely because of increasing off-season  $\text{NO}_3^-$  due to favorable soil conditions in the fall as a result of higher water contents (i.e., decrease in air-filled soil porosity), and a flush of available C as plant roots and high quality (<20 C:N) crop residues start to decompose. A fall tillage experiment conducted on a light textured soil in Denmark reported N<sub>2</sub>O emissions that were 69% greater in the fall following conventional tillage and the incorporation of crop residues as compared to reduced and no-till systems (Mutegi et al., 2010). Best management plans for crop rotations that include legume GMr need not only consider N availabilities to the successive crop, but the potential for over-winter N loss as well.

When GMr residues are soil incorporated, net mineralization is the likely outcome over immobilization because of the high quality (i.e., <20 C:N) organic matter. Soil N mineralization rates and N<sub>2</sub>O flux can increase with the incorporation of GMr residue (Aulakh et al., 1983), but are also shown to reduce cumulative growing season emissions when compared to cropping practices using fertilizers (Osterholz et al., 2014). Furthermore, the flush of microbial activity can deplete soil O<sub>2</sub> concentrations that may promote denitrification. Chirinda et al. (2010a) evaluated emissions of N<sub>2</sub>O from three organic arable crop rotations and one conventional system in Denmark with the hypotheses that organic rotations would have lower N<sub>2</sub>O emissions

because of restricted N availability and that spring emissions would increase following the incorporation of catch crops and grass-clover leys. Both hypotheses were rejected in their study, as cumulative emissions were not different among cropping systems and spring incorporation of plant residues failed to provide any clear pattern in N<sub>2</sub>O emissions.

Although organic systems do not use mineral fertilizers, which are typically associated with increasing N<sub>2</sub>O emissions, the transformation of GMr residue into inorganic N can stimulate N<sub>2</sub>O production as well. Ellert and Janzen (2008) measured higher cumulative (3-yr) N<sub>2</sub>O emissions in a 5-yr crop rotation when wheat and followed three years of continuous alfalfa as compared to a corn-wheat-corn sequence. The use of GMr in rotation may also stimulate N<sub>2</sub>O emissions that occur during spring thaw, especially when incorporated in the fall. Following Sep. alfalfa plowdown in Ontario, Wagner-Riddle et al. (2007) measured N<sub>2</sub>O emissions for Oct. at 1 kg N ha<sup>-1</sup> mo<sup>-1</sup>, but emissions later reached a minimum in Dec, and then increased again at spring thaw (ca. 1.2 kg N ha<sup>-1</sup> mo<sup>-1</sup>). However, no-till and perennial legume GMr could also stimulate N<sub>2</sub>O emissions because of the effect that tillage has on soil pore size and continuity that modifies soil water storage and movement, such as water filled pore space (WFPS) and air entry. Ball et al. (2008) found elevated N<sub>2</sub>O emissions were 1.5 to 35 times higher from no-till treatments as compared to bare soil surfaces that had received inversion tillage. The greater WFPS and greater continuity of soil pores through the profile were said to have a strong control on N<sub>2</sub>O production and emissions, which suggests that emissions were not solely connected to the timing of residue incorporation, but the temporal regulation of water and air in soil pores has a significant control on N<sub>2</sub>O production and emissions. However, there are contrasting results when N<sub>2</sub>O emissions from no-till studies are examined with greater emissions being measured in poorly aerated soils under no-till (Rochette, 2008), and a reduction of N<sub>2</sub>O emissions when no-till is practiced for more than ten years and fertilizer N is placed below the 5-cm soil depth (van Kessel et al., 2013).

## **2.6 Off-Season N<sub>2</sub>O Emissions**

Studies that look at different management strategies of GMr incorporation and the associated N loss from sub-zero soil temperatures are needed (Phillips, 2007), as many of the unknown N losses from cropping systems that rely on organic N sources are suspected to be

from denitrification losses during the dormant season (Phillips, 2008). Because of the reduced availability of C and N substrates or O<sub>2</sub> diffusion to microbial populations (Christensen and Tiedje, 1990; Phillips, 2007; Clark et al., 2009), soil processes responsible for N transformations in arable soils are assumed minimal in sub-zero soil temperatures (Larionova et al., 2007). Risk et al. (2013) reviewed the mechanisms involved in elevated N<sub>2</sub>O emissions during winter and spring thaw as being a combination of: (1) a release of N<sub>2</sub>O produced under a basal soil ice layer that later diffuses out of the soil profile as topsoil thaws (Burton and Beauchamp, 1994; van Bochove et al., 2001), and the off-gassing of N<sub>2</sub>O dissolved in soil water with an increase in temperature (Goodroad and Keeney, 1984; Heincke and Kaupenjohann, 1999), (2) an increase in substrate availability that stimulates the microbial population (*de novo*) (Christensen and Tiedje, 1990; Koponen et al., 2006), as a result of aggregate turnover and the subsequent release of labile organic matter (Bullock et al., 1988; Christensen and Tiedje, 1990; Six et al., 2000; Phillips, 2008), and (3) the activity of specific enzymes and gene expression associated with microorganisms involved in N<sub>2</sub>O production and consumption processes (Wertz et al., 2013; Németh et al., 2014; Tatti et al., 2014).

Considerable soil N<sub>2</sub>O flux has been recorded during spring thaw (Rover et al., 1998; van Bochove et al., 2000b; Jacinthe et al., 2002; Wagner-Riddle et al., 2007; Phillips, 2007; 2008; Wagner-Riddle et al., 2010). Spring thaw accounted for up to 70% of the annual N<sub>2</sub>O emissions from soils (Wagner-Riddle et al., 2008) and were comparable or higher than growing season fluxes from snow-covered soils during winter and spring (van Bochove et al. 2000a). A bi-modal distribution of N<sub>2</sub>O emissions at spring thaw has been described as being an initial physical release of N<sub>2</sub>O (Risk et al., 2014), followed by more rapid and vigorous emissions events newly produced at the soil surface as temperatures fluctuated between the freezing point and 5°C (Wagner-Riddle et al., 2010). Risk et al. (2014) determined that around 75% of the spring thaw N<sub>2</sub>O emission was due to an increase in biological activity at the soil surface (Wagner-Riddle et al., 2010), as compared to emissions that resulted from passive degassing, as described by van Bochove et al. (2001). The extent (i.e., depth, duration, temperature) of soil freezing has been positively related to the size of spring thaw N<sub>2</sub>O emission (Christensen and Tiedje, 1990; Wagner-Riddle et al., 2007; Glenn et al., 2012; Vankoughnett and Henry, 2013); an effect that become especially relevant when a change in climate predicts lower snowfall and more winter

rain events (van Bochove et al., 2001; Henry, 2008), which could lead to a lower snowpack and soil temperatures that would vary more with air temperature.

## **2.7 Soil Profile N<sub>2</sub>O Concentrations**

The composition of soil air at a given point in time is a result of the rate of production and consumption of gases, diffusion of gases through the soil pore spaces, and the partitioning of the gas within the liquid, gaseous, and solid phases of the soil (Farrell et al., 2002). The change in concentration with depth has commonly been used to predict surface flux using the gradient method, as reviewed by Maier and Schack-Kirchner (2014). However, the prediction of effective diffusivity is a major source of uncertainty in these calculations, which are typically derived from air-filled pore-space measurements, without the consideration of how ice at the soil surface affects diffusion or drainage properties. Therefore, in soils that experience freeze–thaw cycles on a daily cycle, the use of this technique is not suitable for predicted surface flux. However, the changes in concentration during freeze–thaw cycles does provide insight into any restrictions to gas diffusion and the potential for N<sub>2</sub>O production and can then be related back to field management or weather patterns. Additionally, Yanai et al. (2011) measured O<sub>2</sub> concentrations at 10 and 30 cm soil depths in conjunction with N<sub>2</sub>O concentrations at similar depths during a thawing event and determined that N<sub>2</sub>O concentrations at depth decreased following a surface N<sub>2</sub>O emission event at same time as the soil O<sub>2</sub> concentration increased to ambient atmospheric levels. A frozen soil layer that restricted soil gas diffusion to the atmosphere and limited O<sub>2</sub> availability in the soil profile was thought to have caused these results.

During freeze–thaw related events, N<sub>2</sub>O is emitted from the soil surface due to changes in solubility with temperature, production near the soil surface, and diffusion from depth (Goodroad and Keeney, 1984; Burton et al., 1997; Risk et al., 2013). Risk et al. (2014) described N<sub>2</sub>O emissions at spring thaw as two distinct events from a silt loam soil near Elora, ON. The first N<sub>2</sub>O emission event was from 12 to 21 Mar. when a total of 3.55 mg N<sub>2</sub>O m<sup>-2</sup> (35.5 g ha<sup>-1</sup>) was measured with a simultaneous N<sub>2</sub>O mass decrease of 14.1 mg N<sub>2</sub>O m<sup>-2</sup> from the soil profile (0–70 cm). The second N<sub>2</sub>O emission event occurred from 21 Mar. to 3 Apr. with a cumulative emission of 12.91 mg N<sub>2</sub>O m<sup>-2</sup>, yet the change in N<sub>2</sub>O mass within the soil profile was only 2.49 mg N<sub>2</sub>O m<sup>-2</sup>. In total, 25% of the accumulated N<sub>2</sub>O within the soil profile was assumed to have

contributed to a surface flux of N<sub>2</sub>O during the first 10 days of spring thaw. A larger N<sub>2</sub>O surface emission event (12.91 mg N<sub>2</sub>O m<sup>-2</sup>) over the next two weeks was then attributed to *de novo* production closer to the soil surface. In a variety of landscape positions in Manitoba, N<sub>2</sub>O accumulated within the soil profile before spring thaw, but profile N<sub>2</sub>O from the lowest position (riparian) were assumed to have been further reduced, as no considerable N<sub>2</sub>O emissions from the surface were recorded (Gao et al., 2014). Although N<sub>2</sub>O has been shown to accumulate within the soil profile over winter, a significant flux at the soil surface during thaw periods does not always occur. These contrasting results highlight the dynamic nature of N<sub>2</sub>O production and release from the soil surface during spring thaw.

## 2.8 Hydrological Soil Properties and N<sub>2</sub>O Emissions

The potential for nitrification and denitrification as related to WFPS were first described by Linn and Doran (1984). Although measures of WFPS give an approximate idea of the aerobic status of a soil, they do not describe the size distribution of soil pores and thus, the overall ability to drain water and accept air at the soil surface. Balaine et al. (2013) described a positive relationship between maximum N<sub>2</sub>O emissions and soils air-entry potential ( $r^2 = 0.96$ ), which implied that high N<sub>2</sub>O emissions are not just associated with high denitrification potential, but that emissions occur when soil pores are not completely blocked with water. Similarly, three landscape positions (e.g., ditch, near-ditch, mid-field) that varied in bulk density (0.7–1.2 g cm<sup>-3</sup>) and total C (11.1–15.6 g kg<sup>-1</sup>) from a poorly drained, silty soil in Maryland had different levels of water content, but a similar range of matric potential (–3.75 kPa) at the time of maximum N<sub>2</sub>O emissions from intact soil columns (Castellano et al., 2010). A matric potential of –3.75 kPa represents a pore radius of 39.58 µm, which indicates that maximum N<sub>2</sub>O flux occurred as pores of this size have completely drained of water. Comparing field operations from different areas and climates then becomes challenging, without a clear understanding of how the hydrological properties of a soil compare. Greater daily N<sub>2</sub>O emissions (440 to 740 g N ha<sup>-1</sup> d<sup>-1</sup>) following precipitation events and N fertilization were measured from fine textured soils in southern Manitoba (Glenn et al., 2012) when compared to a field (150 g N ha<sup>-1</sup> d<sup>-1</sup>; Wagner-Riddle et al., 2007) that had actually received greater N fertilization, but had better drainage and less available C. Similarly, sharp peaks in N<sub>2</sub>O emissions under wet no-till conditions were produced by

greater water contents as compared to tilled soil, without a further reduction to  $N_2$  (Ball et al., 2008). Finally, two soils with similar total porosity, but one with greater macro-porosity (i.e., 3000  $\mu\text{m}$ ) than the other also had different  $N_2O$  emission patterns (van der Weerden et al., 2012). Larger pores with greater connectivity provided faster soil water infiltration and lower cumulative emissions, which allowed more air to occupy soil pores, and thus, a lower potential for denitrification and  $N_2O$  emission. Models that predict the pore size and continuity in soils are expected to become increasingly important to our understanding of the mechanisms that control gas production, transport, and release to the atmosphere (Ball, 2013). Soil water content and the size and distribution of soil pores have a strong control on the aeration status of a soil, which is ultimately controlled by incoming precipitation and a soil's ability to infiltrate water.

The pattern and extent of soil freezing will be affected by tillage, because of the change in soil pores and a modification of surface conditions, such as vegetation, that will trap more snow and insulate the soil from freezing and thawing cycles (Ling and Zhang, 2004). During soil freezing, smaller pores will freeze last because of osmotic and matric potentials that keep water from freezing (Harrysson Drotz et al., 2009). In contrast, smaller pores will thaw before larger pores because of the smaller volume of water and amount of latent heat needed to complete the phase change (Kane et al., 2001). The amount of drainage becomes a good indicator of the soil's ability to transmit water and gas at the soil surface because water acts as a gas diffusion barrier from the soil to the atmosphere when all soil pores at the soil surface are filled with ice (Cherkauer and Lettenmaier, 1999; Ireson et al., 2013; Watanabe et al., 2013). When soil experiences freeze-thaw dynamics, soil pore characteristics and hydrological properties might have an even greater control on  $N_2O$  production and related emissions. The timing of tillage in the fall will also have an effect on the size and continuity of soil pores during the off-season (Parkin et al., 2013), which will affect not only the rate and extent of soil freezing, but the rate and timing of soil thawing and the subsequent rapid infiltration of water from the surface and through the profile.

## **2.9 Dissolved $N_2O$**

The first discussions about the amount of dissolved  $N_2O$  that exits a field in drainage tiles were initiated by Dowdell et al. (1979) who estimated that  $0.25 \text{ kg N ha}^{-1}$  could be lost from 250

mm of precipitation (Nov.–Mar.) in the U.K. During their study period, the amount of  $\text{N}_2\text{O}$  lost in drainage tiles equaled surface emissions measured at the site; they concluded that during periods of high  $\text{N}_2\text{O}$  concentrations and rapid drainage, dissolved  $\text{N}_2\text{O}$  losses are significant. Since then, various other studies have measured or estimated relatively low concentrations in drainage water and have therefore determined this to be an insignificant pathway for N loss for most agricultural areas. Nitrous oxide is highly soluble in cold water (Heincke and Kaupenjohann, 1999) and becomes a likely pathway for N losses when arable soils are equipped with sub-surface drainage systems that can contribute to global agriculture related GHG emissions (Baulch et al., 2011). Roper (2009) measured differences in dissolved  $\text{N}_2\text{O}$  collected from drainage tiles from a sandy soil under continuous and no-till treatments. No difference in tillage treatment (till vs. no-till) was observed over a 1.5 yr sampling period, yet there was a similar seasonal variation in dissolved  $\text{N}_2\text{O}$  ( $\mu\text{g N}_2\text{O L}^{-1}$ ) in drainage water from both treatments that was influenced by a similar pattern of freeze–thaw events. The concentration of  $\text{NO}_3^-$  in collected drainage water during the same sampling times did not show the same variation, but cumulative loads of  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$  were significantly correlated. The difference in  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$  concentrations highlighted the contrasting soil environment that is experienced in soils that experience freeze thaw–events, but the positive relationship between cumulative losses of each suggests that the volume and pattern of drainage events had a control on overall N losses. The contribution of accumulated soil gas to surface emissions and the movement in drainage water is controlled by physical soil properties such as air-entry values, and gradients of water potential and temperature (Smith et al., 2003; Blagodatsky and Smith, 2012).



### **3. OFF-SEASON SOIL N<sub>2</sub>O EMISSIONS AS AFFECTED BY THE TIMING OF GREEN MANURE TILLAGE**

#### **3.1 Preface**

Synchronizing soil N availability with plant uptake is the goal for systems that use green manures (GMr) for soil fertility because an improvement to N efficiency also minimizes environmental N losses to air (N<sub>2</sub>O) and drainage water (NO<sub>3</sub><sup>-</sup>). Biennial GMr crops generate considerable amounts of N in above- and below-ground biomass that is maintained through the off-season, prior to cash crop planting. Spring incorporation is thought to have the lowest risk for off-season N losses, but the ideal soil conditions needed for inversion tillage may not align with all of the other field activities occurring in the spring. As a result, producers may incorporate the GMr crop early or late into the fall when soil conditions and time are more suitable. A considerable proportion of annual N<sub>2</sub>O emissions have been measured during spring thaw; thus, it is of particular interest to understand how fall or spring GMr management affects off-season N<sub>2</sub>O emissions. The length, depth, and frequency of soil freezing and thawing has been suggested to be a good predictor of N<sub>2</sub>O emissions, while in other studies there is no clear edaphic property that predicts N<sub>2</sub>O emissions at spring thaw. The goal of this study is to measure N<sub>2</sub>O emissions from a red clover GMr crop through two consecutive off-seasons with respect to the timing of GMr incorporation and the removal of aboveground biomass. Because N<sub>2</sub>O emissions measured during spring thaw can be significant, there is a focus on understanding the impact of soil freeze/thaw dynamics on these emission events.

### 3.2 Abstract

Off-season  $\text{N}_2\text{O}$  emissions can be a major proportion of annual emissions, but little is known about the timing of these emissions from leguminous green manures (GMr), especially during spring thaw. Over two years, we measured off-season (Sep.–June)  $\text{N}_2\text{O}$  emissions from a biennial GMr–spring wheat rotation in Atlantic Canada where GMr was incorporated in Sep. (Early Fall; EF), Nov. (Late Fall; LF), and May (Spring; SP). An additional treatment removed GMr biomass as summer hay before Nov. incorporation (Hayed Late Fall; HLF). Off-season emissions ranged from 0.41 to 0.95 kg  $\text{N}_2\text{O}\text{-N ha}^{-1}$  in Year 1 and 0.26 to 1.2 kg  $\text{N}_2\text{O}\text{-N ha}^{-1}$  in Year 2 with the largest daily emissions of 45.34 and 59.82 g  $\text{N}_2\text{O}\text{-N ha}^{-1} \text{ d}^{-1}$  measured from SP and the mean of HLF and LF during spring thaw, respectively. All three fall incorporated clover treatments led to similar cumulative  $\text{N}_2\text{O}$  emissions during the off-season, but EF had 45 to 78% greater fall (Sep.–Jan.) emissions than all other treatments in Year 1 and 2, respectively. Delaying GMr incorporation until May (Spring; SP) produced different cumulative (Sep.–June)  $\text{N}_2\text{O}$  emissions when compared to all fall treatments in both years, however these differences were in different directions. In Year 1, SP was nearly twice as large as compared to fall treatments (950 vs. 494 g  $\text{N}_2\text{O}\text{-N ha}^{-1}$ ), while fall treatments were 4.3 times as great as SP (262 g  $\text{N}_2\text{O}\text{-N ha}^{-1}$ ) in Year 2. Treatments with colder and deeper soil frost (i.e., LF, HLF) had the highest emissions at spring thaw in Year 2, while the interaction of soil warming and the placement of GMr residue may have had a stronger influence in Year 1. However, there was a consistent relationship between years when peak  $\text{N}_2\text{O}$  emissions at spring thaw occurred during the rapid increase in soil temperature and water drainage. The season and timing of GMr incorporation may modify these properties that control the timing of emissions, which suggests that hydrological properties through the entire soil profile have a significant control on  $\text{N}_2\text{O}$  emissions during spring thaw.

### 3.3 Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) is a persistent greenhouse gas (GHG) that had an atmospheric concentration of 324.2 ppb in 2011, which was an increase of 5-ppb since 2005 (Hartmann et al., 2013). Although the contribution of  $\text{N}_2\text{O}$  to overall radiative forcing is small (e.g., 6.0–6.24%; Butterbach-Bahl et al., 2013; Myhre et al., 2013), the fact that  $\text{N}_2\text{O}$  is the primary ozone

depleting substance in the atmosphere (Cicerone, 1989; Ravishankara et al., 2009; Wuebbles, 2009) further emphasizes the need to understand how management decisions affect N<sub>2</sub>O emissions. The production of N<sub>2</sub>O as a result of agricultural activities contributes roughly 30 to 65% of atmospheric concentrations (Butterbach-Bahl et al., 2004; Ambus et al., 2011) and these emissions are primarily produced through denitrification and nitrification soil processes (Butterbach-Bahl et al., 2004; Pennock et al., 2006). Emissions of N<sub>2</sub>O are typically associated with the rate and timing of N inputs, soil tillage, increased soil water, and freeze–thaw events (Burton et al., 1997; Gregorich et al., 2008; Risk et al., 2013), yet the proportion of N<sub>2</sub>O produced during denitrification as compared to N<sub>2</sub> is a function of C and NO<sub>3</sub><sup>−</sup> availability, in addition to the availability of oxygen in the soil (Butterbach-Bahl and Dannenmann, 2011).

Organic agriculture relies heavily on the use of animal manures and the use of legume species as cover crops or GMr, yet the contribution of N<sub>2</sub>O from legume residues is not well understood (Rochette and Janzen, 2005). Increased N<sub>2</sub>O emissions in GMr systems are due to the rapid release of inorganic N (N<sub>min</sub>) from high quality (i.e., C:N < 20) legume residues (Baggs et al., 2000; Huang et al., 2004; Rochette and Janzen, 2005; Mitchell et al., 2013). This has led to a greater interest in determining best management plans for potato and other high-value crops in cool maritime climates (Sharifi et al., 2009; Lynch et al., 2012; Snowdon et al., 2013; Wertz et al., 2013), which could include managing the timing and season of GMr incorporation. Fall incorporation of GMr would allow for an earlier spring planting date in areas with a shorter growing season (Carter, 1992), but the absence of a growing crop following fall GMr plowdown poses a greater risk for N<sub>2</sub>O emissions (Phillips, 2008; Ball et al., 2014). Spring GMr incorporation is commonly adopted with the intent to minimize N losses (Baggs et al., 2000; Lynch et al. 2012; Ball et al., 2014). However, spring incorporation can delay N uptake of the succeeding crop due to immobilization or slow microbial N transformations from excess soil water and cool soil temperatures in some areas (Aulakh et al., 1991; Cookson et al., 2002; Lahti and Kuikman, 2003; Thorup-Kristensen et al., 2003; Woodley et al., 2014). Alternatively, N mineralization may occur well in advance of cash crop planting (Chapter 5), which may lead to increased N losses and diminished yields.

In addition to fall or spring GMr incorporation, other management options include delaying fall incorporation until soil temperatures are cool enough to slow soil mineralization and nitrification processes, as described for red clover–potato rotations (Sanderson and

MacLeod, 1994; Sanderson et al., 1999) and spring cereals in Atlantic Canada (Carter, 1994). Nitrification was inhibited in soil incubated at 2 or 5°C due to the slow acclimation of nitrifying organisms in cold soils (Cookson et al., 2002), which would reduce the risk of N losses from the reduction in  $\text{NO}_3^-$  intensity (Burton et al., 2008). The ability to limit nitrification through delayed GMr incorporation in the fall is a promising management option that could reduce  $\text{N}_2\text{O}$  emissions through the off-season. An alternative cropping strategy could involve cutting or grazing aboveground biomass as forage prior to fall incorporation, with the possibility for supplemental manure additions in the spring (Brozyna et al., 2013; Froseth et al., 2014).

The carryover of large amounts of organic C and N in GMr biomass through the winter is of particular concern for off-season  $\text{N}_2\text{O}$  emissions, especially at spring thaw, when  $\text{N}_2\text{O}$  emissions have been reported to be a considerable proportion of annual emissions (Duxbury et al., 1982; Rover et al., 1998; Wagner-Riddle et al., 2007; Matzner and Borken, 2008; Johnson et al., 2010; Yanai et al., 2011; Glenn et al., 2012). Both freezing and thawing intensity at the soil surface, measured as accumulated degree hours ( $\Sigma\text{DH}$ ), have been suggested as controls over spring thaw  $\text{N}_2\text{O}$  emissions (Christensen and Tiedje, 1990; Wagner-Riddle et al., 2007; Glenn et al., 2012; Maas et al., 2013). Furthermore, Brumme et al. (1999) suggested that the duration of event-based emissions, such as those at spring thaw, are controlled by the soil environment, which is modified by soil re-wetting and freeze–thaw events, whereas the pattern of background emissions through the year relates to site-specific conditions, such as surface characteristics (e.g., bare or vegetated soil), and drainage properties. The interaction of soil physical and biological processes responsible for  $\text{N}_2\text{O}$  production are intimately connected by water filled pore space and soil diffusivity, which have been identified as good predictors of  $\text{N}_2\text{O}$  emissions (Ball et al., 2008; Rees et al., 2013; Petersen et al., 2013; Balaine et al., 2013). Although soil freezing and thawing may occur most intensely at the soil surface, it is clear that these soil dynamics will have a significant control on water and gas exchange through the entire soil profile. There needs to be consideration of the effects that GMr incorporation during different times of the year has on biological, chemical, and physical soil properties and processes as they relate to off-season  $\text{N}_2\text{O}$  emissions.

Incorporating GMr with inversion tillage affects soil water infiltration and holding capacity because of a change in the size and distribution of soil pores. Therefore, tillage alters the amount of free water at a particular soil temperature (Parkin et al., 2013), which then can alter

the pattern and extent of soil freezing and thawing dynamics. Thus, when GMr tillage is conducted in the fall, there are physical effects on soil properties and processes that could also affect denitrification and N<sub>2</sub>O emissions. First, the exposure of a bare soil surface decreases the soil albedo (Williams et al., 2009) and lessens the ability to trap snow that insulates the soil surface from freezing and thawing events (Iwata et al., 2011), altering thermal and water dynamics known to control N<sub>2</sub>O production and the subsequent release to the atmosphere, especially at spring thaw (Henry, 2008; Blankinship and Hart, 2012). Secondly, inversion plowing disrupts preferred pathways (i.e., macropores), which have a major control on water and gas exchange (Carter, 1996; Kay and VandenBygaart, 2002; Abdollahi and Munkholm, 2014). Finally, a temporary (Strudley et al., 2008) reduction in bulk density (Kay and VandenBygaart, 2002) by loosening soil through the tillage depth (Carter, 1996) reduces the occurrence of anaerobic conditions needed for denitrification. Manipulating soil water and surface vegetation with fall tillage is an example of how field management and cropping patterns can decrease or increase freeze–thaw related soil N<sub>2</sub>O emissions (Dietzel et al., 2011).

Studies are needed that look at cool season N losses from legumes grown as GMr and forage, with respect to their timing of incorporation (Phillips, 2007; 2008; Clark et al., 2009) because cool season N<sub>2</sub>O emissions are not measured or assumed to be minimal in cool soil temperatures (Morkved et al., 2006; Phillips, 2008). Decisions for cropping patterns that include GMr in the rotation should consider how GMr incorporation affects N<sub>2</sub>O emissions through the whole year (Aulakh et al., 1991; Rochette and Janzen, 2005; Wagner-Riddle et al., 2007; Brozyna et al., 2013), rather than only taking measurements during the growing season. Furthermore, the effect of GMr management should also consider snow levels that are predicted to diminish in temperate northern latitudes and the subsequent increase in soil freeze/thaw frequency as a result of climate change (Groffman et al., 2001). The objective of this two-year study was to measure soil N<sub>2</sub>O emissions (Sep. to June) from red clover GMr that differed in management by the timing of incorporation from early fall to spring. Inorganic N content, CO<sub>2</sub> emissions (e.g., microbial activity), and the effect of air and soil temperatures were also tracked to help interpret the timing and magnitude of N<sub>2</sub>O emissions through the off-season.

### 3.4 MATERIALS AND METHODS

#### 3.4.1 Field site description and experimental design

All field data were collected at the Brookside experimental site for organic agriculture at Dalhousie University's Agricultural Campus, Truro, Nova Scotia, Canada (45°23'24.72" N lat; -63° 15'16.15" W long) from the off-season (Sep.10<sup>th</sup>–June 1<sup>st</sup>) over two years. Soil at the Brookside experimental site is a well to imperfectly drained sandy loam (Orthic Humo-Ferric Podzol) from the Woodville soil series (Webb et al., 1991). Soil pH (H<sub>2</sub>O) at the beginning of the experiment was measured at 6.3±0.3 and total soil C and N was 19.8±3.9 and 1.7±0.3 kg m<sup>-2</sup>, respectively, from the top 0 to 15 cm of soil. Mean annual temperature is 5.8°C and annual precipitation is 1224 mm, which includes a mean snowfall of 214 cm. Annual total degree-days (>5°C) average 1700 and the frost-free period is 99 d. Drainage at the site was further improved by the installation in 2008 of subsurface drainage tiles at a depth of 0.8 m.

This study was part of a larger project that is looking at fertility management practices for legumes grown as GMr in red clover (*Trifolium pratense* L.) – spring wheat (*Triticum aestivum* L.) crop sequences within a 4-yr rotation. The crop sequence consisted of soybeans (Year 1), two years of red clover (Years 2, 3), and spring wheat (Year 4). The overall experiment was designed in a randomized block design with four main plots (16 x 75 m) and four sub-plots (16 x 16 m) representing all four phases of the crop rotation. Each treatment and phase was then replicated over three blocks. A diagram of the field plot layout is found in Appendix 1.

We focused on the Year 3 clover phase (GMr) prior to the spring wheat phase (Year 4) of the rotation. Treatments differed in management and timing of GMr incorporation using a moldboard plow (Table 3.1). Treatments included: (1) Clover incorporated in Sep. (Early Fall; EF), (2) Clover incorporated in Nov., with aboveground clover biomass removed (Hayed Late Fall; HLF), (3) Clover incorporated in Nov. (Late Fall; LF), and (4) Clover undisturbed until incorporation in May (Spring; SP).

**Table 3.1.** Description and timing of GMr treatments at the experimental site 2011–2013.

| Treatments                        | Activity   | Year 1       | Year 2       |
|-----------------------------------|--|--------------|--------------|
| Hayed Late Fall                   | Clover biomass harvested as hay                      | 23 June 2011 | 18 June 2012 |
| Early Fall, Late Fall, and Spring | Clipped clover, residue remained on the soil surface | 23 June 2011 | 18 June 2012 |
| Hayed Late Fall                   | Clover biomass harvested as hay                      | 18 Aug. 2011 | 31 July 2012 |
| Early Fall, Late Fall, and Spring | Clipped clover, residue remained on the soil surface | 18 Aug. 2011 | 31 July 2012 |
| Early Fall                        | Red clover incorporation                             | 17 Sep. 2011 | 18 Sep. 2012 |
| Hayed Late Fall and Late Fall     | Red clover incorporation                             | 2 Nov. 2011  | 7 Nov. 2012  |
| Spring                            | Red clover incorporation                             | 2 May 2012   | 1 May 2013   |

### 3.4.2 Clover biomass and quality

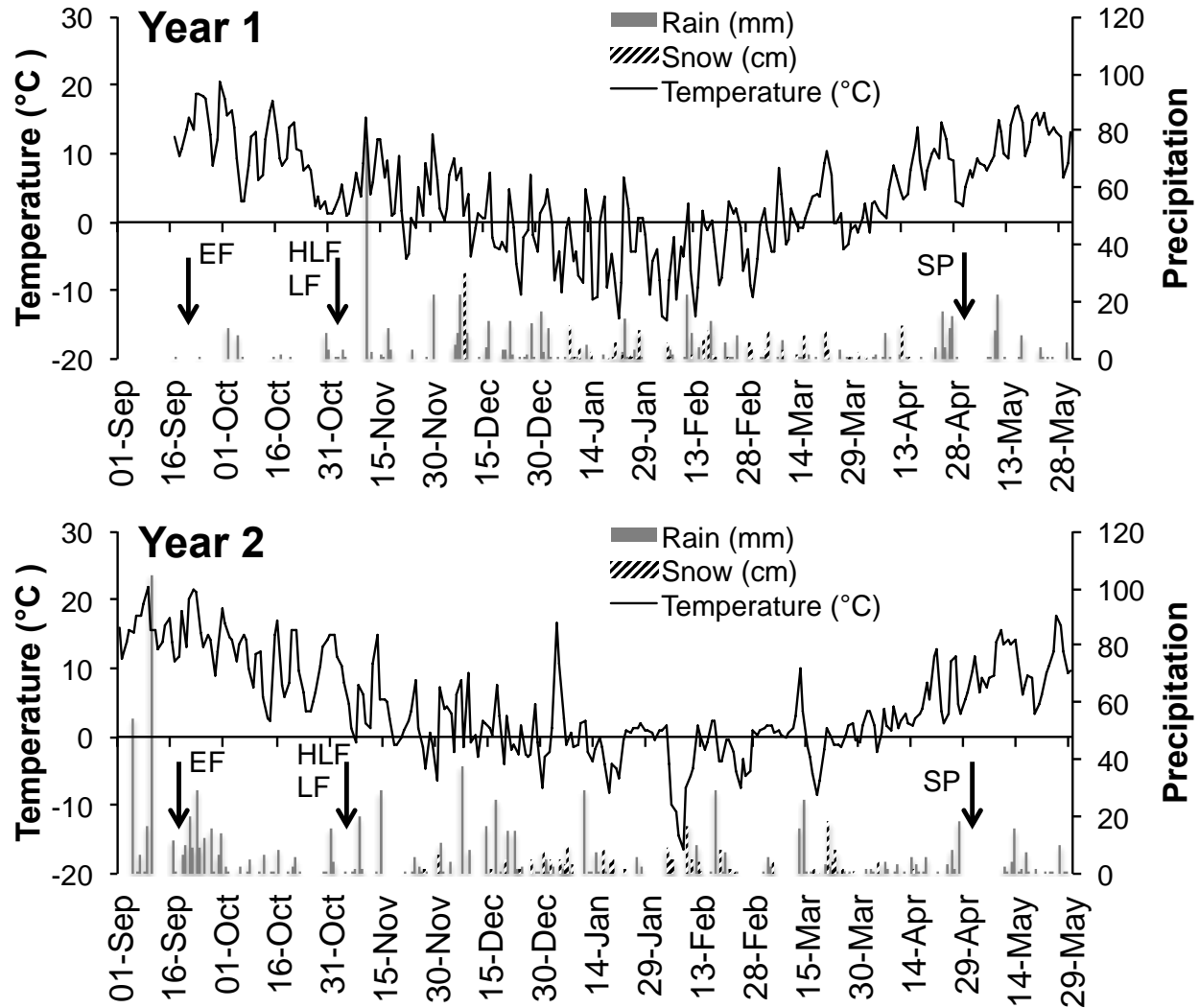
Clover was either clipped (i.e., EF, LF, and SP) or removed (i.e., HLF) twice during the growing season (Table 3.1) to keep clover in its vegetative state. Clover biomass was measured among all treatments prior to clover clipping or removal (HLF only) by weighing a 1.5 m x 16m strip from the center of each plot with a Haldrup forage harvester (J. Haldrup, Løgstor, Denmark) equipped with a biomass scale. A sub-sample was then dried for dry matter (DM) determination and the quality of clover biomass was determined by C and N concentrations using a CN analyzer (Vario Max, Elementar, Hanau, Germany).

### 3.4.3 Climate, soil temperature and water content monitoring

Soil temperature and volumetric water content were measured using a single 5TE sensor (Decagon Devices, Pullman, WA, USA) in each plot ( $n=12$ ) for measurements at 15 cm and at the soil surface (0–5 cm) from both plowed and vegetated surfaces in each block. Sensors were installed at the 15 cm depth by excavating a narrow hole with a tree planter's shovel in each plot and inserting perpendicular to the surface in the undisturbed sidewall of the narrow hole. Each sensor had a soil volume of influence of 715 cm<sup>3</sup>.

Air temperature and precipitation were measured on-site using a shielded thermocouple (Campbell Scientific, Logan, UT, USA) and tipping bucket with hourly measurements logged to a CR200X data logger (Campbell Scientific, Logan, UT, USA) (Fig. 3.1). Daily mean soil temperatures were calculated from hourly measurements logged to an EM-50 data logger

(Decagon Devices, Pullman, WA, USA). Accumulated degree hours ( $\Sigma DH$ ) is the sum of Freezing Degree Hours ( $\Sigma FDH$ ) and Thawing Degree Hours ( $\Sigma TDH$ ) for air and soil that were calculated by summing the daily degree hours below and above 0°C (Wagner-Riddle et al., 2007; Yanai et al., 2011; Glenn et al., 2012).



**Fig. 3.1.** Weather at the Brookside research site in Truro, NS, Canada from Sep. to June over two years. Vertical arrows show the timing of clover incorporation for Early Fall (EF), Hayed Late Fall (HLF) and Late Fall (LF) combined, and Spring (SP).

#### 3.4.4 Soil inorganic N

Surface soil (15 cm) was sampled typically on the same day as gas sampling by compositing 10 soil samples per plot taken with a handheld probe (2 cm i.d.) in Year 1 only. Soil



samples were immediately frozen in an on-site freezer until they were thawed and then extracted for  $N_{\min}$  with 0.5 M  $K_2SO_4$  (3:1, liquid:soil) for 1-hr and filtered through glass fiber filter paper following the method of Keeney and Nelson (1982) before  $NH_4^+$  and  $NO_2^- + NO_3^-$  were analyzed colorimetrically with an auto-analyzer (Technicon Instruments Corporation, Tarrytown, NY, USA).

#### 3.4.5 $N_2O$ and $CO_2$ surface emissions

A vented, non-steady-state PVC chamber was used for  $N_2O$  and  $CO_2$  measurements. Chambers were 20 cm diameter and 15 cm in height, which created a chamber volume of 4.7 L and a surface area of 314.16 cm<sup>2</sup>. Reflective and insulated material covered the outside of the chamber. Permanent collars measuring 10 cm in height were placed five to seven cm in the soil and were only removed during field tillage. All plants were removed from inside the collars. There was no change to the gas sampling protocol when collars were filled with snow. However, chambers were placed on top of the snow if snow levels were higher than the collars, which occurred on two sampling dates in Jan. of Year 2.

Emissions of  $N_2O$  and  $CO_2$  were measured at least twice per calendar month from Sep. 10<sup>th</sup> to June 1<sup>st</sup> in Year 1 (2011–12) and Year 2 (2012–13), with the exception of the month of Feb. in Year 1 when collars filled with ice and only a single measurement was taken. An additional sampling date was included in both Jan. and Feb. of Year 2, as well as more frequent sampling after legume residue incorporation in Sep. and Nov. and near the initial thaw periods (8 Mar. 2012 and 14 Mar. 2013). Spring thaw was signaled by the beginning of rapid water infiltration, as measured by on-site tipping buckets used to quantify outflow from tile drains. Emissions of  $CO_2$  from the soil surface are interpreted as heterotrophic respiration (i.e., plant roots and microbes) and used as a proxy for microbial activity, not to assess C cycling and storage in soils. Cumulative  $N_2O$  emissions were calculated from Sep. to June in Year 1 (274-d) and Year 2 (273-d) on an individual chamber basis by linear interpolation between sampling dates, using the assumption that gas flux measured on a sample date represents the daily average flux. When a sampling frequency of 14 to 21 d is used, Parkin (2008) reported that these estimates were –43 to 64% of the “true” cumulative emissions, which were estimated with automated chambers four times each day. Flux measurements are dependent on the underlying temporal variability across a particular site during that point in time. Therefore, the temporal

pattern of N<sub>2</sub>O–N emissions as a result of differing GMr management is of greater interest than the comparison of cumulative flux between these systems.

Air from the chamber headspace was sampled by withdrawing 20 mL of air through a rubber septum at the top of the chamber at regular intervals using plastic disposable syringes and transferred to 12 mL pre-evacuated (67 pa) vials (Labco Exetainer, High Wycombe, U.K.). A total of 24 chambers (2 sub-samples per plot) were sampled over 4 time intervals (0, 10, 20 and 30 min) for a total of 96 samples per sampling date. Four replicates of 1.1 µL L<sup>-1</sup> N<sub>2</sub>O gas standards were taken on each sampling date to ensure the reliability of the storage and handling of the gas samples (Burton et al., 2008).

Gas analysis was performed using a Varian Star 3800 Gas Chromatograph (Varian, Walnut Creek, CA) fitted with an electron capture detector (ECD), thermal conductivity detector (TCD), and a Combi-PAL Autosampler (CTC Analytics, Zwingen, Switzerland). The Combi-PAL injects 2.5 mL into the gas chromatograph to fill two 0.5-mL sampling loops that load gas onto ECD and TCD/FID flow streams. The ECD was operated at 380°C, 90% Ar, 10% CH<sub>4</sub> carrier gas at 20 mL min<sup>-1</sup>, Haysep N 80/100 pre-column (0.32 cm diameter×50 cm length) and Haysep D 80/100 mesh analytical columns (0.32 cm diameter×200 cm length) in a column oven operated at 70°C. The pre-column was used in combination with a four-port valve to remove water from samples. The TCD was operated at 130°C, pre-purified He carrier gas at 30 mL min<sup>-1</sup>, Haysep N 80/100 mesh (0.32 cm diameter ×50 cm length) pre-column followed by a Porapak QS 80/100 mesh (0.32 cm diameter×200 cm length) analytical column maintained at 70°C.

As described by Burton et al. (2008), fluxes ( $F_c$ ) of N<sub>2</sub>O and CO<sub>2</sub> (g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> and kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>) were calculated using the following equation (Hutchinson and Livingston, 1993):

$$F_c = dC / dt \cdot V_c M_{\text{mol}} / (A V_{\text{mol}}) \quad [1]$$

where  $dC/dt$  is the rate of change in N<sub>2</sub>O or CO<sub>2</sub> concentration (mol mol<sup>-1</sup> h<sup>-1</sup>),  $A$  is the surface area (m<sup>2</sup>) of the chamber,  $V_c$  is the total volume (L) of the chamber (i.e., chamber plus exposed collar),  $M_{\text{mol}}$  is the molar mass of N<sub>2</sub>O (g mol<sup>-1</sup>) and  $V_{\text{mol}}$  is the volume of a mole of N<sub>2</sub>O (L mol<sup>-1</sup>) inside the chamber corrected for temperature using the ideal gas law. The flux value of  $dC/dt$  was calculated using the simple linear regression of gas concentrations versus time of the deployment period. The slope of the line multiplied by the chamber volume and

division by the chamber surface area will result in flux units of  $\mu\text{L gas m}^{-2} \text{ min}^{-1}$ . All flux data are presented as  $\text{g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ , or  $\text{kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ .

### 3.4.6 Statistical analysis

Daily  $\text{N}_2\text{O}$  and  $\text{CO}_2$  flux measurements on each sample date were evaluated for Treatment, Time and the interaction of Treatment x Time effects using repeated measured using a mixed model split-plot approach that treated sub-samples ( $n=6$ ) as the random effect. Monthly cumulative  $\text{N}_2\text{O}$  emissions were evaluated using a similar repeated measures model, where Month was the repeated factor. Cumulative  $\text{N}_2\text{O}$  emissions for the entire season in each year were evaluated for the effect of Treatment using a mixed model where sub-samples was the random effect. Sub-samples were at least 10 m apart in each experimental unit, which allowed us to assume that any spatial auto-correlation would be minimal (Ball et al., 2007). Clover biomass from the first and second clipping were summed together and mean residue quality (i.e., C:N) were tested for differences in Treatment, Year, and their interaction using a mixed model where block was the random factor. The effect of Treatment, Time, and the interaction of Treatment x Time on soil  $\text{N}_{\text{min}}$  in Year 1 only and 15 cm soil  $\Sigma\text{DH}$  in both years were analyzed using a mixed model and treating block ( $n=3$ ) as the random effect. Differences between years for monthly and annual air temperatures were determined with a two-tailed t-test. Treatment means were separated with Tukey's post-hoc test and a test of the simple main effects (slices) when the interaction of Treatment and Time was significant. Soil  $\text{N}_{\text{min}}$ ,  $\text{N}_2\text{O}$ , and  $\text{CO}_2$  data were log transformed to help satisfy the assumptions of equal variance and normality, while untransformed means and standard errors are presented. All effects were considered significant when  $p \leq 0.05$ , and statistical tests were performed using JMP software (SAS Institute Inc. Cary, NC, USA).

## 3.5 RESULTS

### 3.5.1 *Clover biomass and quality*

Results in Year 1 refer to field data collected from 1 Sep. 2011 to 31 May 2012 and Year 2 represents the same dates in 2012 and 2013. Prior to the experiment, visual estimates of GMr plant cover in 2<sup>nd</sup> yr red clover plots confirmed that 90 to 95% of the plot area was covered by red clover, while the remaining plant biomass was a mix of grasses and annual weedy forbs (data not shown). There was no difference in aboveground clover biomass (Table 3.2) across treatments ( $p= 0.28$ ) or years (0.07), nor was the interaction significant ( $p= 0.69$ ). Mean aboveground clover biomass at the end of the growing season over the 2-yr study had a range of 8.7 to 10.6 Mg ha<sup>-1</sup> (dry wt.), which represented an input of organic-N of 238 to 311 kg ha<sup>-1</sup>. Biomass quality was assessed by C:N ratio and was not different among treatments ( $p= 0.91$ ), nor was the interaction with Year significant ( $p= 0.47$ ). The effect of Year was strongly significant ( $p= 0.0004$ ), yet the biological significance of having small average differences in the C:N ratio GMr tissue (14.2 as compared to 15.6 in Year 1 and 2, respectively) may be considered minor, but does imply a 10% higher GMr biomass N content in Year 1 over Year 2.

**Table 3.2.** Clover aboveground biomass (Mg ha<sup>-1</sup> dry wt.) and quality during the growing seasons prior to incorporation in Year 1 and 2.

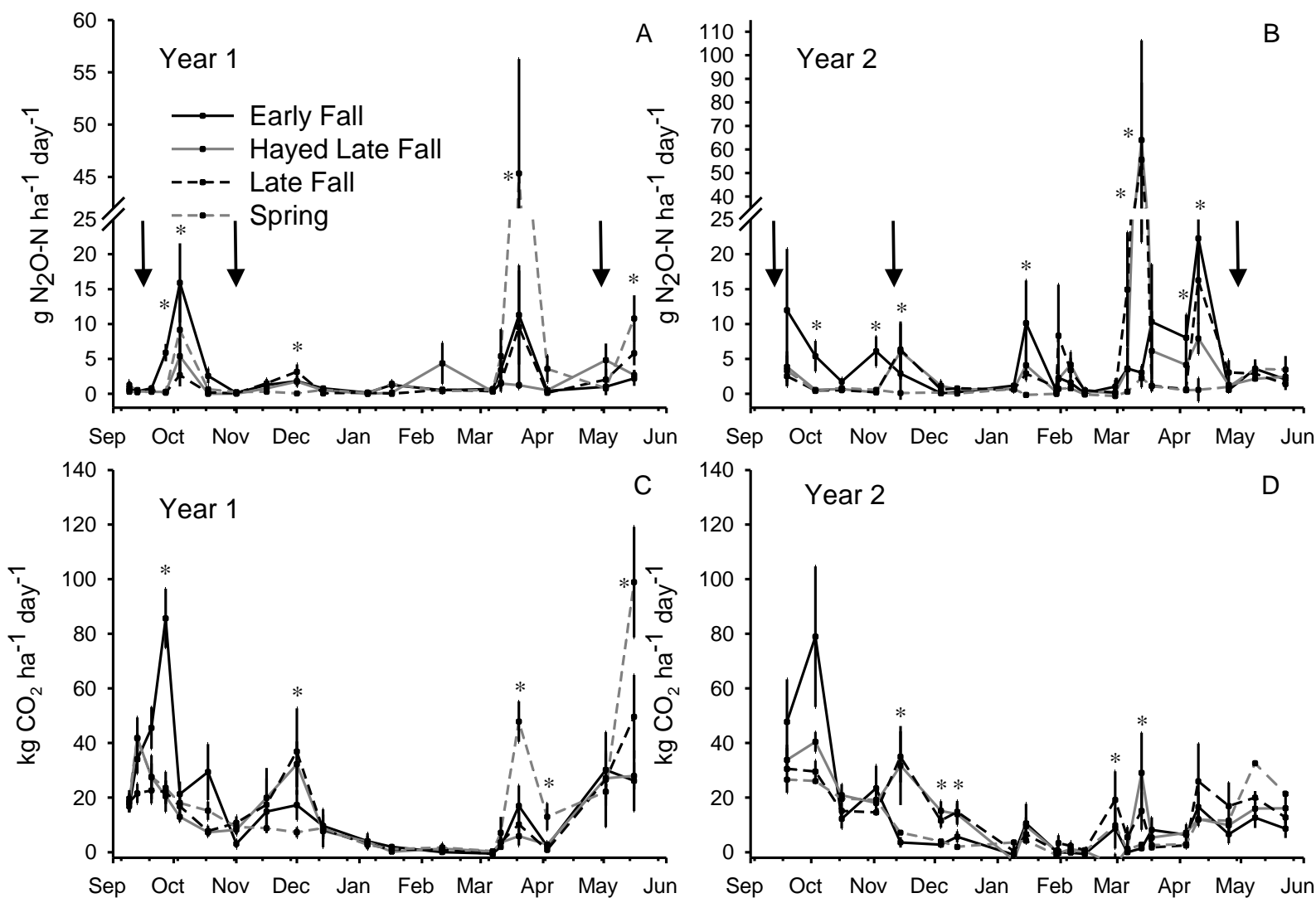
| Treatments                 | Biomass (DM Mg ha <sup>-1</sup> )     |              | Biomass quality (C:N) |             |
|----------------------------|---------------------------------------|--------------|-----------------------|-------------|
|                            | Year 1                                | Year 2       | Year 1                | Year 2      |
| Early Fall                 | 9.05 (0.09) <sup>†</sup>              | 10.58 (0.36) | 14.6 (0.67)           | 15.6 (0.7)  |
| Hayed Late Fall            | 8.71 (0.24)                           | 9.00 (0.94)  | 14.4 (0.68)           | 15.4 (0.82) |
| Late Fall                  | 8.77 (0.83)                           | 9.29 (0.57)  | 14.1 (0.67)           | 15.4 (1.07) |
| Spring                     | 9.3 (0.57)                            | 9.93 (0.74)  | 13.7 (0.53)           | 16.1 (0.72) |
| <b>Source of variation</b> | Probability ( <i>p</i> ) <sup>‡</sup> |              |                       |             |
| Treatment (T)              | 0.279                                 |              | 0.908                 |             |
| Year (Y)                   | 0.074                                 |              | <b>0.0004</b>         |             |
| TxY                        | 0.688                                 |              | 0.472                 |             |

<sup>†</sup> Values in brackets represent the SEM (*n*=3).

<sup>‡</sup> Bolded values indicate a significant difference at the *p* ≤ 0.05 level of probability.

### 3.5.2 N<sub>2</sub>O emissions

The Treatment and Time interaction was significant (*p* < 0.0001) in Year 1 (Fig. 3.2 A), and three sample dates between 27 Sep. 2011 and 4 Oct. 2011 all had greater N<sub>2</sub>O emissions from the EF treatment as compared to all other treatments. However, on 1 Dec. 2011 the greatest N<sub>2</sub>O emissions, although small in magnitude, were measured from LF incorporated clover (3.15 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>) and the smallest from SP plots (0.05 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>). The largest emission event from any treatment was measured on 20 Mar. 2012 from the SP treatment (45.34 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>) and the smallest on this day was measured from the HLF treatment (1.24 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>). Two weeks following SP clover incorporation (16 May 2012), the SP treatment had the largest N<sub>2</sub>O emission of 10.8 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> and the least from the EF and HLF (2.45 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>).



**Fig. 3.2.** Soil emissions of  $\text{N}_2\text{O}$  (A, B) and  $\text{CO}_2$  (C, D) from fall incorporated red clover in Year 1 and 2. Significant differences ( $\alpha = 0.05$ ) in  $\text{N}_2\text{O}$  and  $\text{CO}_2$  across treatments for each sample date are indicated with “\*”. Vertical arrows (from left to right) show the timing of Early Fall (EF), Late Fall (LF) and Hayed Late Fall (HLF) combined, and Spring (SP) clover incorporation in Year 1 and 2. Error bars represent the SEM ( $n=6$ ).

In Year 2, the Treatment x Time interaction was also significant ( $p < 0.0001$ ) and showed a similar temporal pattern to Year 1 (Fig. 3.1 B). Early Fall clover incorporation produced greater  $\text{N}_2\text{O}$  emissions on 3 Oct. 2012 and 2 Nov. 2012 as compared to all other treatments, while Nov. clover incorporation (7 Nov. 2012) produced the largest  $\text{N}_2\text{O}$  emissions in the LF and HLF treatments ( $6.19 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ) when compared to SP ( $0.11 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ). During the middle of winter, the largest emissions were measured from EF ( $10.12 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ) and the smallest from SP plots ( $-0.16 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ) on 15 Jan. 2013. The peak of spring thaw in Year 2 was on 13 Mar. 2013 with the greatest  $\text{N}_2\text{O}$  emissions being measured from both the LF and HLF treatments ( $59.82 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ) as compared to both the EF and SP clover incorporated treatments that had a mean of  $2.65 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ . At the beginning of Apr., EF emissions ( $8.05 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ) were nearly four times emissions from all other treatments ( $1.75 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ). There was another large emission event on 10 Apr. 2013, when both EF and LF clover incorporation treatments had the largest  $\text{N}_2\text{O}$  emissions with 22.27 and 16.28  $\text{g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ , respectively, and the smallest emissions were measured from the SP treatment ( $0.57 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ).

### 3.5.3 $\text{CO}_2$ emissions

The effect of Treatment across all sample dates (Fig. 3.1 C) was not significant ( $p = 0.104$ ) in Year 1, but the interaction with Time was ( $p < 0.0001$ ). Emissions of  $\text{CO}_2$  were larger in EF plots on 27 Sep. 2011 with  $85.67 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ , as compared to an average of  $22.07 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$  from the other three treatments. Both LF and HLF treatments had  $\text{CO}_2$  emissions that were 4.7 times as great as compared to SP ( $7.32 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ) on 1 Dec. 2011, while EF was intermediate ( $17.31 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ). Undisturbed plots (SP) in Year 1 had the greatest  $\text{CO}_2$  emissions ( $47.89 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ) on 20 Mar. 2012 as compared to both the LF and HLF treatments ( $8.04 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ), while EF was intermediate with  $16.96 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ . Two weeks later on 3 Apr. 2012, SP plots again had 6.5 times the  $\text{CO}_2$  emissions as compared to the mean of all other treatments ( $2.01 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ). On the last sampling date in Year 1 (16 May 2012),  $\text{CO}_2$  emissions from SP incorporated clover were again twice as large when compared to LF ( $49.64 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ), and the smallest from EF and HLF plots ( $27.08 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ).

The Treatment and Time interaction was also significant ( $p = 0.0003$ ) in Year 2 (Fig. 3.1 D). Between 14 Nov. 2012 and 12 Dec. 2012, the largest  $\text{CO}_2$  emissions among treatments were

measured in both LF and HLF treatments, with average emissions across the three sample dates of  $20.35 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$  when compared to the smallest from both EF and SP ( $4.2 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ). In contrast to the pattern of  $\text{CO}_2$  emissions at spring thaw in Year 1, LF and HLF treatments had emissions that were 11 times greater than both EF and SP treatments ( $2.14 \text{ kg CO}_2 \text{ ha}^{-1} \text{ d}^{-1}$ ); following the same pattern among treatments for  $\text{N}_2\text{O}$  on 13 Mar. 2013.

#### 3.5.4 Cumulative $\text{N}_2\text{O}$

Across all treatments, cumulative  $\text{N}_2\text{O}$  emissions were 48% lower in Year 1 as compared to Year 2 with the highest  $\text{N}_2\text{O}$  emissions ( $p=0.02$ ) being measured from the SP treatment ( $950.09 \text{ g N}_2\text{O-N ha}^{-1}$ ) in Year 1, and smallest  $\text{N}_2\text{O}$  emissions from LF and HLF ( $444.35 \text{ g N}_2\text{O-N ha}^{-1}$ ) treatments (Table 3.3). Early Fall had intermediate values of  $599.91 \text{ g N}_2\text{O-N ha}^{-1}$ . In contrast, the smallest ( $p=0.047$ ) cumulative  $\text{N}_2\text{O}$  emissions in Year 2 were measured from the SP treatment ( $261.95 \text{ g N}_2\text{O-N ha}^{-1}$ ) when compared to all three fall incorporated clover treatments ( $1113.91 \text{ g N}_2\text{O-N ha}^{-1}$ ). When cumulative emissions are analyzed by fall (Sep.–Dec.) and spring (Mar.–June) seasons, delaying GMr incorporation until Nov. did reduce fall  $\text{N}_2\text{O}$  emissions as compared to EF, but emissions did increase in the spring, which effectively canceled out any prior reductions. In Year 1, 50% of the seasonal  $\text{N}_2\text{O}$  emissions from the EF treatment were measured in the fall, as compared to 14, 21, and 24% from the SP, HLF, and LF treatments, respectively. In comparison, EF incorporated clover had 46% of the total  $\text{N}_2\text{O}$  emissions measured in the spring of Year 2, as compared to 65, 70, and 53% from HLF, LF, and SP treatments, respectively.



**Table 3.3.** Monthly and cumulative N<sub>2</sub>O emissions from red clover (Sep.-June) over two years.

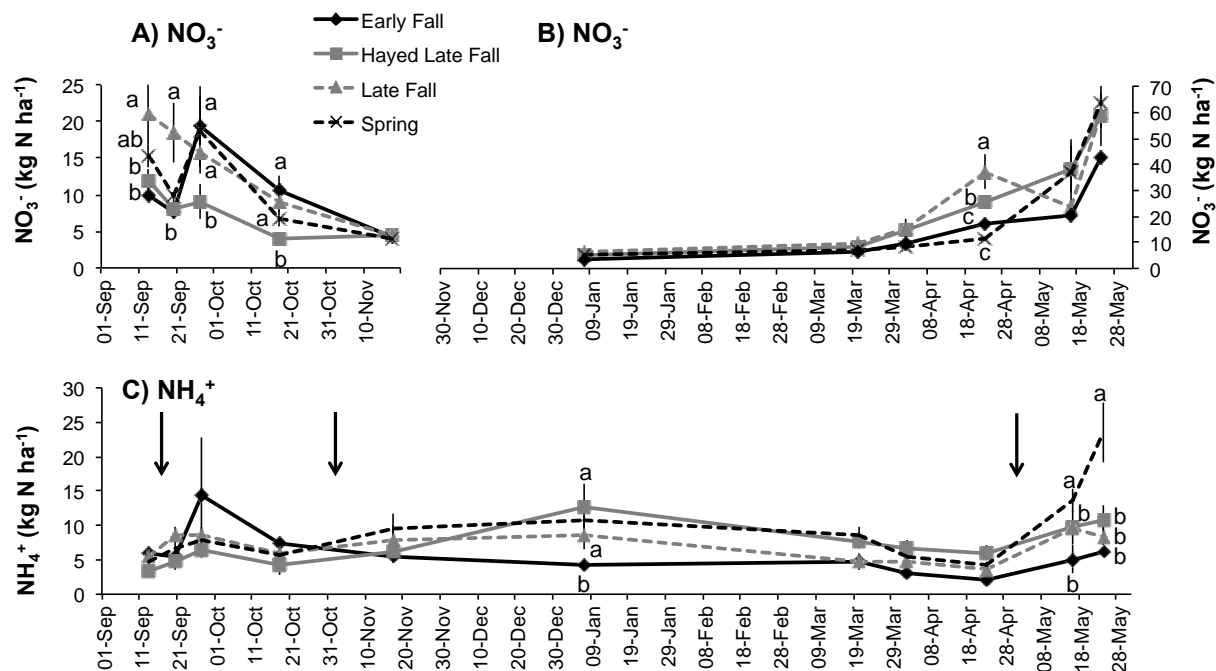
| Months            | Year 1        | Treatments  |                 |                 |                 | Year 2        | Treatments  |                  |                  |                 |
|-------------------|---------------|---|-----------------|-----------------|-----------------|---------------|---|------------------|------------------|-----------------|
|                   |               | Early Fall  | Hayed Late Fall | Late Fall       | Spring          |               | Early Fall  | Hayed Late Fall  | Late Fall        | Spring          |
|                   | $p \leq 0.05$ | ----- g N <sub>2</sub> O-N ha <sup>-1</sup> month <sup>-1</sup> ----- |                 |                 |                 | $p \leq 0.05$ | ----- g N <sub>2</sub> O-N ha <sup>-1</sup> month <sup>-1</sup> ----- |                  |                  |                 |
| <b>Sep</b>        | 0.019         | 60.42 a <sup>†</sup>  | 14.20 b         | 12.15 b         | 14.46 b         | 0.263         | 273.82  | 147.46           | 64.06            | 75.80           |
| <b>Oct</b>        | <0.001        | 196.02 a  | 53.62 b         | 30.07 b         | 99.30 b         | 0.026         | 120.05 a  | 18.09 b          | 14.40 b          | 21.53 b         |
| <b>Nov</b>        | 0.089         | 33.43   | 25.02           | 46.13           | 7.82            | 0.015         | 101.42 a  | 105.30 a         | 114.76 a         | 9.55 b          |
| <b>Dec</b>        | 0.083         | 27.95   | 22.50           | 25.36           | 12.16           | 0.24          | 13.80   | 26.30            | 24.05            | 7.50            |
| <b>Jan</b>        | 0.466         | 25.75   | 22.37           | 6.40            | 21.74           | 0.003         | 124.47 a  | 55.66 a          | 53.93 a          | 7.00 b          |
| <b>Feb</b>        | 0.324         | 18.90   | 90.49           | 15.17           | 19.07           | 0.005         | 22.20 a   | 32.12 a          | 35.88 a          | 2.80 b          |
| <b>Mar</b>        | 0.002         | 150.88 b  | 44.31 b         | 101.61 b        | 437.00 a        | <0.001        | 201.44 a  | 537.08 a         | 440.94 a         | 30.93 b         |
| <b>Apr</b>        | 0.252         | 23.40   | 72.77           | 31.42           | 51.38           | <0.001        | 298.52 a  | 129.18 a         | 215.64 a         | 30.37 b         |
| <b>May</b>        | 0.131         | 63.16   | 129.66          | 145.46          | 287.16          | 0.941         | 64.79   | 50.87            | 55.49            | 76.47           |
| <b>Cumulative</b> | <b>0.02</b>   | <b>599.91 ab</b>  | <b>474.95 b</b> | <b>413.75 b</b> | <b>950.09 a</b> | <b>0.047</b>  | <b>1220.52 a</b>  | <b>1102.07 a</b> | <b>1019.15 a</b> | <b>261.95 b</b> |

<sup>†</sup>Means within rows with the same lower case letter for each month and cumulative emissions in each year are not significantly different among treatments at the  $p \leq 0.05$  level of probability. Significantly different cumulative emissions are bolded.

### 3.5.5 Soil inorganic N ( $N_{min}$ )

Soil  $N_{min}$  (0–15 cm) was evaluated using a repeated measures analysis (Fig. 3.2) on samples taken during Year 1 (Sep.–June) only. The overall effect of treatment on  $NO_3^-$  ( $kg\ NO_3^- -N\ ha^{-1}$ ) was not significant ( $p= 0.18$ ), yet the Treatment x Time interaction was ( $p< 0.0001$ ). Late Fall plots had the highest  $NO_3^-$  content on 13 Sep. 2011 as compared to the lowest in EF and HLF, while SP was intermediate. One week later (20 Sep. 2011), the highest  $NO_3^-$  content was measured from the LF treatment ( $18.51\ kg\ NO_3^- -N\ ha^{-1}$ ) as compared to all other treatments that had a mean value of  $8.57\ kg\ NO_3^- -N\ ha^{-1}$ . Hayed Late Fall plots had a soil  $NO_3^-$  content that was 51% smaller on 27 Sep. 2011 when compared to all other treatments ( $17.95\ kg\ NO_3^- -N\ ha^{-1}$ ). The lowest  $NO_3^-$  contents were again measured from HLF plots ( $3.98\ kg\ NO_3^- -N\ ha^{-1}$ ) on 18 Oct. 2011, when compared to all other treatments ( $8.8\ kg\ NO_3^- -N\ ha^{-1}$ ). During spring thaw, the highest  $NO_3^-$  content was from LF ( $37.06\ kg\ NO_3^- -N\ ha^{-1}$ ), followed by HLF ( $25.27\ kg\ NO_3^- -N\ ha^{-1}$ ), as compared to both EF and SP treatments ( $14.42\ kg\ NO_3^- -N\ ha^{-1}$ ).

The content of  $NH_4^+$  at the soil surface (0–15 cm) over all sampling times was significantly greater ( $p= 0.04$ ) from the SP treatment ( $9.15\ kg\ NH_4^+ -N\ ha^{-1}$ ), as compared to the least in EF ( $5.8\ kg\ NH_4^+ -N\ ha^{-1}$ ). On 7 Jan. 2012, EF had a  $NH_4^+$  content ( $kg\ NH_4^+ -N\ ha^{-1}$ ) that was nearly 2.5 times lower than all other plots with a mean of  $10.72\ kg\ NH_4^+ -N\ ha^{-1}$ . At the end of the study period (23 Apr. 2012), EF had the lowest  $NH_4^+$  content ( $2.08\ kg\ NH_4^+ -N\ ha^{-1}$ ) when compared to all other treatments ( $4.64\ kg\ NH_4^+ -N\ ha^{-1}$ ). Following SP clover incorporation on 2 May 2012,  $NH_4^+$  in SP incorporated clover plots went from 13.62 to 23.53 on 16 and 24 May, respectively which was 68 and 181% greater than all other treatments.



**Fig. 3.3.** Soil  $\text{NO}_3^-$  (A, B) and  $\text{NH}_4^+$  (C) content from the 15 cm soil depth from Sep. to June in Year 1.  $\text{NO}_3^-$  is divided into 2 panels due to the different scale used from Sep. to early Nov. (A) and late Nov. through May (B). Arrows represent the timing of clover incorporation (from left to right) for Early Fall (EF), Late and Hayed Late Fall (LF, HLF), and Spring (SP) treatments. Means with the same lower case letter are not significantly different at the  $p \leq 0.05$  level of probability. Error bars represent the SEM ( $n=3$ ).

### 3.5.6 Air temperature

A two-tailed  $t$ -test revealed no significant difference ( $p=0.16$ ) in the seasonal (Dec. to Apr.) average air temperature between the two years of study. Mean air temperatures were  $-1.9^\circ\text{C}$  for Year 1 and  $-2.8^\circ\text{C}$  during Year 2 (Table 3.4). Although the small change in average temperature seems insignificant, the difference in temperature was actually a result of 1582 more freezing hours ( $\Sigma\text{FDH}$ ) in Year 2, and 1294 less overall thawing hours ( $\Sigma\text{TDH}$ ). December mean air temperatures were on average  $1^\circ\text{C}$  warmer in Year 1, as compared to Year 2, yet not different ( $p=0.16$ ). However, the sum of freezing and thawing hours ( $\Sigma\text{DH}$ ) produced a positive value in Year 1 (448) and a negative value ( $-313$ ) in Year 2. Air temperatures were significantly ( $p=0.05$ ) lower in Jan. of Year 2 ( $-6.8^\circ\text{C}$ ), as compared to Year 1 ( $-3.7^\circ\text{C}$ ). Mean temperatures in Feb. were not different ( $p=0.6$ ), which was also reflected by similar warming and cooling degree hours. During the month of Mar., mean air temperatures were not different ( $p=0.77$ ), but temperature means and associated accumulated degree hours ( $\Sigma\text{DH}$ ) during the first half of Mar.

revealed considerably more freezing conditions in Year 1. In contrast, there were more warming conditions ( $\Sigma DH$ ) from Mar. 16 to 31 in Year 1 as compared to Year 2.

**Table 3.4.** Average daily air temperature from December to March in Year 1 and 2, Freezing Degree Hours ( $\Sigma FDH$ ), Thawing degree hours ( $\Sigma TDH$ ), and their sum ( $\Sigma DH$ ).

| Dates                    | Mean Daily Air Temperature |             | $p \leq 0.05$ | Air Freezing Degree Hours ( $\Sigma FDH$ ) |               | Air Thawing Degree Hours ( $\Sigma TDH$ ) |             | $(\Sigma DH)^{\dagger} = (\Sigma FDH) + (\Sigma TDH)$ |              |
|--------------------------|----------------------------|-------------|---------------|--|---------------|---|-------------|---|--------------|
|                          | Year 1                     | Year 2      |               | Year 1                                     | Year 2        | Year 1                                    | Year 2      | Year 1  | Year 2       |
| <b>Dec<sup>‡</sup></b>   | <b>0.6</b>                 | <b>-0.4</b> | <b>0.16</b>   | <b>-1445</b>                               | <b>-1819</b>  | <b>1893</b>                               | <b>1506</b> | <b>448</b>  | <b>-313</b>  |
| 1–15                     | 2.8                        | 0.5         |               | -270                                       | -830          | 1290                                      | 1005        | 1020  | 175          |
| 16–31                    | -1.5                       | -1.3        |               | -1175                                      | -989          | 603                                       | 501         | -572  | -488         |
| <b>Jan</b>               | <b>-3.7</b>                | <b>-6.8</b> | <b>0.05</b>   | <b>-3535</b>                               | <b>-5673</b>  | <b>812</b>                                | <b>640</b>  | <b>-2723</b>  | <b>-5033</b> |
| 1–15                     | -3.3                       | -5.4        |               | -1595                                      | -2171         | 412                                       | 210         | -1183   | -1961        |
| 16–31                    | -4.0                       | -8.0        |               | -1941                                      | -3502         | 399                                       | 430         | -1542   | -3072        |
| <b>Feb</b>               | <b>-4.9</b>                | <b>-4.2</b> | <b>0.6</b>    | <b>-3532</b>                               | <b>-3242</b>  | <b>370</b>                                | <b>325</b>  | <b>-3162</b>  | <b>-2917</b> |
| 1–14                     | -7.5                       | -6.4        |               | -2614                                      | -2202         | 109                                       | 65          | -2505   | -2137        |
| 15–28                    | -2.0                       | -2.3        |               | -918                                       | -1040         | 262                                       | 260         | -656  | -780         |
| <b>Mar</b>               | <b>0.4</b>                 | <b>0.2</b>  | <b>0.77</b>   | <b>-1794</b>                               | <b>-1154</b>  | <b>1956</b>                               | <b>1266</b> | <b>162</b>  | <b>112</b>   |
| 1–15                     | -1.1                       | 1.6         |               | -1219                                      | -185          | 659                                       | 775         | -560  | 590          |
| 16–31                    | 1.8                        | -1.2        |               | -575                                       | -969          | 1297                                      | 491         | 722   | -478         |
| <b>Total<sup>§</sup></b> | <b>-1.9</b>                | <b>-2.8</b> | <b>0.37</b>   | <b>-10306</b>                              | <b>-11888</b> | <b>5031</b>                               | <b>3737</b> | <b>-5275</b>  | <b>-8151</b> |

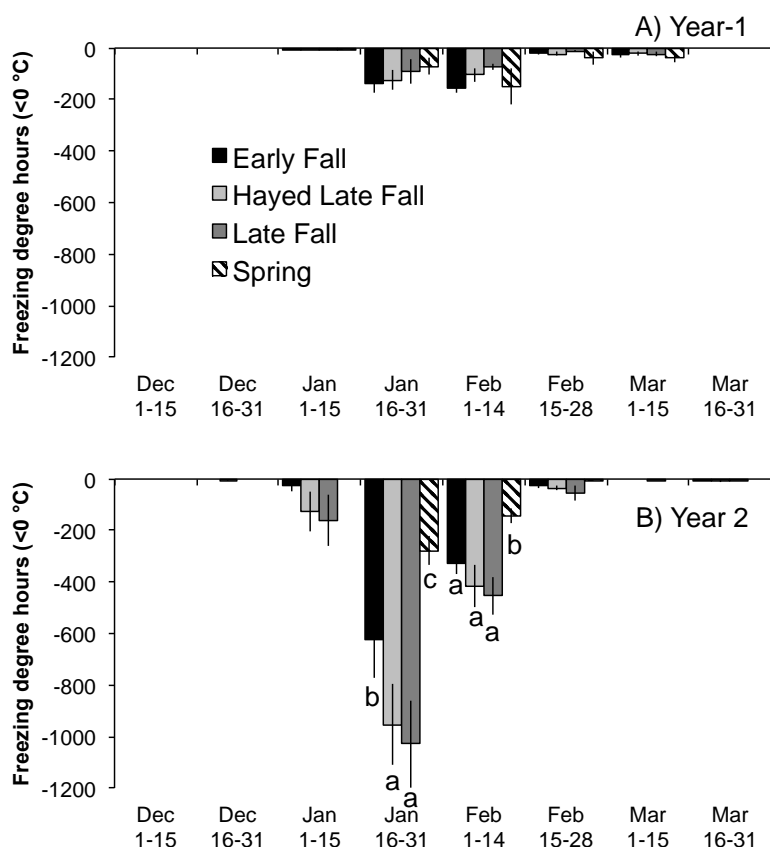
<sup>†</sup> The sum of freezing and thawing hours ( $\Sigma DH$ )

<sup>‡</sup> Monthly and total temperatures means for Year 1 and 2 are bolded and bi-weekly means are below.

<sup>§</sup> Total refers to the seasonal (Dec.–Apr.) average temperature.

### 3.5.7 Soil temperature

Accumulated freezing hours were evaluated twice monthly for differences among treatments at the 15 cm soil depth (Fig. 3.3). During the first year of study, there were no differences in  $\Sigma FDH$  among treatments ( $p= 0.7$ ), nor was the interaction with Time significant ( $p= 0.74$ ). In Year 2 there was nearly four times the number of freezing hours in LF and HLF plots, and twice as many in the EF treatment as compared to SP, yet overall was marginally significant ( $p= 0.07$ ). In the second half of Jan. there were 713 more freezing hours recorded in LF and HLF plots, and 344 more in EF as compared to SP. At the beginning of Feb. there were 255 more freezing hours in the three fall incorporated clover plots as compared to SP.



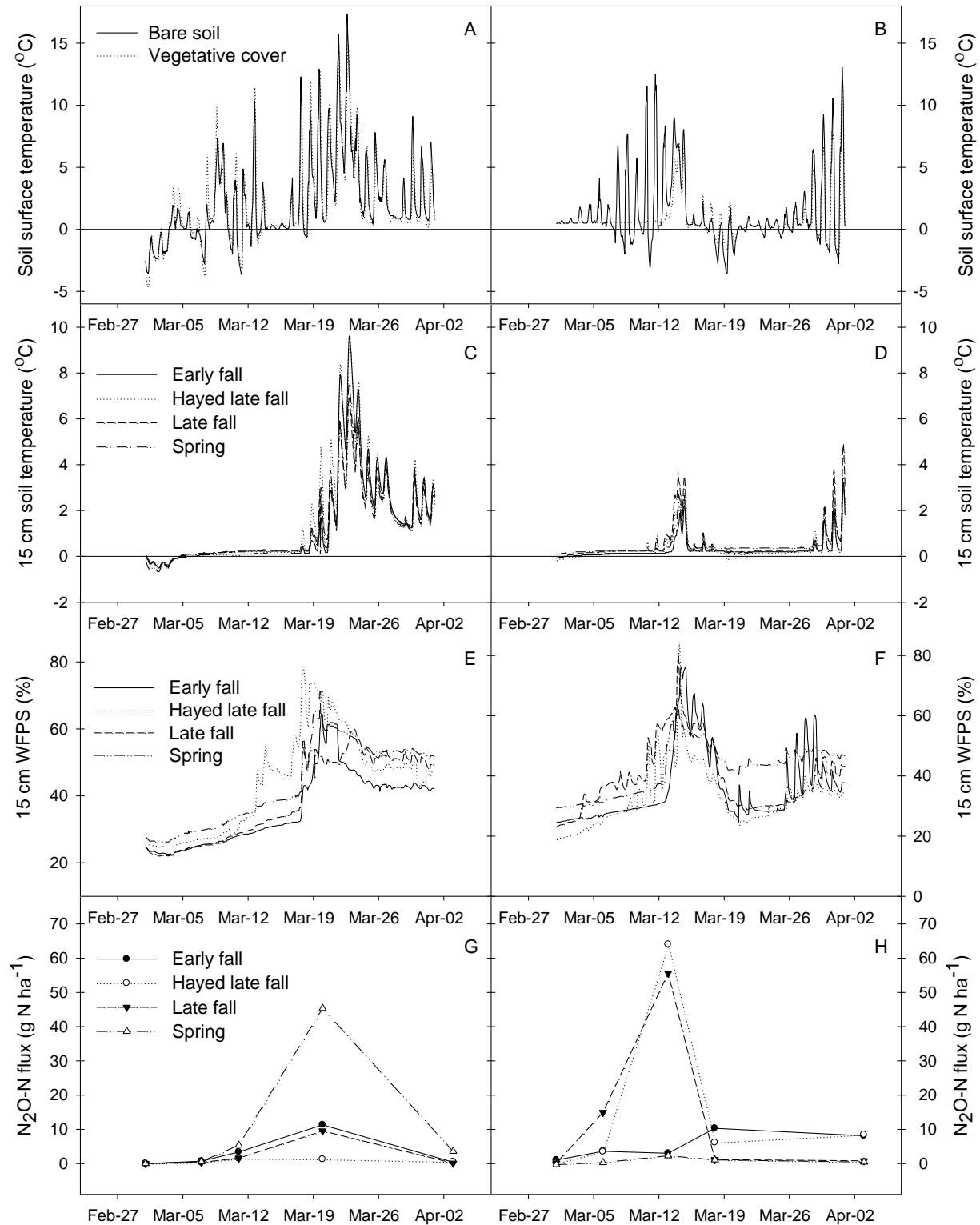
**Fig. 3.4.** Freezing degree hours ( $\Sigma$ FDH) from the 15 cm soil depth during Year 1 (A) and Year 2 (B). Treatments with the same letter are not significantly different at the  $p \leq 0.05$  level of probability. Error bars represent the SEM ( $n=3$ ).

### 3.5.8 Freeze thaw effects on the timing of $\text{N}_2\text{O}$ emissions

The rapid increase in soil temperature and water filled pore space (WFPS) at the 15 cm soil depth during spring thaw in March (Fig. 3.4) occurred as the highest daily  $\text{N}_2\text{O}$  emissions are measured from the soil surface in both years of study. The increase in soil temperature at 15 cm is likely a result of both convection of solar energy from the soil surface and the mass transfer of heat from water at the soil surface, an indication that soil water infiltration is no longer restricted by ice. Soil temperatures remained at or near  $0^{\circ}\text{C}$  in all treatments through the beginning of Mar., which is a signal that soil water is in the process of making a complete phase change to liquid (Outcalt et al., 1990). During this time, macropores ( $> 75 \mu\text{m}$ ) that are largely responsible for water and gas exchange through the profile start to melt before smaller pores and rapid infiltration begins. The higher WFPS in the HLF treatment (Fig. 3.4 E) at the peak emission event that was measured in all other treatments in Year 1 (Fig. 3.4 G) is evidence of  $\text{N}_2\text{O}$

reduction to  $N_2$ , which was more likely from natural field variability, than an effect of treatment because the only change in this treatment, as compared to LF, was the removal of aboveground biomass in two cuts of hay from HLF the previous summer, which does not adequately explain the rapid and greater increase in soil water content and the observed decline in  $N_2O$  emissions.

Surface soil temperatures (0–5 cm) were measured from both bare soil (fall tilled treatments) and a vegetated surface (spring plowed clover; SP). Sensors used to measure surface temperatures had a variable measurement depth due to the frequent action of freezing and thawing and the production of ice lenses that moved the sensor through the measurement depth (0–5 cm), but are still a good measurement of surface temperature dynamics between treatments. An increase in the magnitude and frequency of daily air freezing and thawing cycles (Fig 3.1) created similar surface conditions between the two cover types in Year 1 and surface soil temperatures did not vary through spring thaw. In contrast, variation in surface temperatures between bare soil (fall plowed) and vegetated cover (spring plowed) through the thawing period is likely due to the observed greater snow trapping and the higher albedo of vegetation as compared to bare soil.



**Fig. 3.5.** The timing of N<sub>2</sub>O emissions during “spring thaw” (Feb.–Apr.) Years-1 and -2 with soil surface temperature (A, B), soil temperature at 15 cm soil depth of (C, D), water filled pore space (WFPS) at 15 cm soil depth (E, F), and N<sub>2</sub>O flux (G, H). Surface temperature is from the 0–5 cm soil depth in bare (fall plowed) and cover (Spring; SP) plots.

### 3.6 DISCUSSION

#### 3.6.1 *Timing of GMr incorporation and N<sub>2</sub>O emissions*

We measured a large amount of year-to year variation in cumulative off-season (Sep.–June) N<sub>2</sub>O emissions with 48% more N<sub>2</sub>O emissions in Year 2, as compared to Year 1 and a range of 0.26 to 1.22 kg N<sub>2</sub>O-N ha<sup>-1</sup> between all treatments and years. However, the timing and proportion of off-season emissions was consistent over the two years; 60% of cumulative off-season emissions (Sep.–June) were measured in the spring following spring thaw (Mar.–June) and 26 to 31% in the fall (Sep.–Dec.). An exception to this overall trend was the EF treatment that had an average of 65 and 66% greater fall emissions than all other treatments in Year 1 and 2, respectively. Katayangi and Hatano (2012) compared winter to annual cumulative N<sub>2</sub>O emissions from croplands in 13 studies without specifying the winter measurement period in each study and determined that winter N<sub>2</sub>O emissions can range from 0 to 19 kg N ha<sup>-1</sup> and be anywhere from 0 to 93% of all annual emissions. Using micrometeorological techniques, Wagner-Riddle et al. (2007) reported off-season N<sub>2</sub>O emissions (Nov.–Apr.) over five different years and two management regimes of 0.42 and 2.9 kg N ha<sup>-1</sup>, as compared to 0.08 to 0.89 kg N ha<sup>-1</sup> over the same measurement period in our study. Typically, peak off-season emissions are attributed to the actions of soil freezing and thawing (e.g., Matzner and Borken, 2008), or melting snow (Risk et al., 2013), and have been collectively referred to as freeze–thaw related emissions. In our study, the greatest daily flux events occurred during the month of Mar., but this was not consistent in all treatments; spring thaw emissions in Mar. ranged from 9 to 49% of the cumulative off-season (Sep.–June) emissions across all treatments and years.

The biggest difference in N<sub>2</sub>O emissions at spring thaw when compared over two years was in the SP treatment. Spring incorporated clover differed from all three fall incorporated clover treatments in both years, although in an unexpected direction in Year 1. Spring incorporation had N<sub>2</sub>O emissions that were 91% larger ( $p=0.02$ ) than the three fall incorporated treatments in Year 1, but in Year 2, N<sub>2</sub>O emissions were only 24% of the three fall treatments.



Baggs et al. (2000) found SP incorporation of a 2-yr-old grass–white clover sward produced 0.24 kg N<sub>2</sub>O-N ha<sup>-1</sup> from Mar. to June, which is less than the emissions measured in our study, but comparable. Wagner-Riddle et al. (2007) reported 32 to 48% greater off-season (Nov.–Apr.) N<sub>2</sub>O emissions in fall tilled as compared to no-till in three of the five study years and attributed these differences to greater insulation at the soil surface from crop residues at the soil surface in no-till plots and less soil freezing. Results in Year 2 of our study were similar to Wagner-Riddle et al. (2007), but not in Year 1.

Over the entire off-season period, there were no differences in cumulative N<sub>2</sub>O emissions from any of the three fall incorporated clover treatments in either year. However, the timing of these emissions differed and occurred as bursts of N<sub>2</sub>O immediately after incorporation for the EF treatment (Sep.–Oct.) of both years, and an increase following Nov. incorporated treatments in Year 2 (Table 3.2). Similar studies that investigated off-season N<sub>2</sub>O emissions from the timing of fall GMR incorporation are rare; however, it is not uncommon for N<sub>2</sub>O emissions to increase following tillage (Mutegi et al., 2010), fall manure application (Rochette et al., 2004), or alfalfa plowdown in Sep. (Wagner-Riddle and Thurtell, 1998). It was surprising that the removal of roughly 9 Mg ha<sup>-1</sup> as dry above-ground clover biomass in the HLF treatment did not significantly change cumulative N<sub>2</sub>O emissions when compared to LF, or EF treatments. There are a few conclusions that can be drawn from this result: (1) a large proportion of clover N is retained within the root and crown portions of the plant, (2) when clover residue is mulched at the soil surface, rather than removed as hay, much of the N is lost, or (3) N<sub>2</sub>O emissions are limited by other factors than just the availability of substrate (i.e., placement, drainage, temperature effects on enzyme and microbe activity). However, it was discussed earlier that the reduced spring thaw emissions were as a result of reduced O<sub>2</sub> conditions, as indicated by the increases in WFPS in Year 1 during spring thaw (Fig. 3.4 E) and the subsequent, likely reduction of N<sub>2</sub>O to N<sub>2</sub> needs to be considered when interpreting these results.

The measurement of root and microbial respiration (i.e., CO<sub>2</sub> flux) following clover incorporation is a good indicator of residue decomposition and microbial activity. These measurements provide some insight on the timing of the microbial N demand and the supply of substrate (i.e., NO<sub>3</sub><sup>-</sup> and labile C) needed during denitrification. Increases in N<sub>2</sub>O emissions were linked to CO<sub>2</sub> emissions, especially following clover incorporation in both years and when clover residue was left on the soil surface (SP) at spring thaw in Year 1. While increased microbial

activity and the possibility for limited O<sub>2</sub> availability as a result can promote denitrification (Burton et al., 2012), it is important to note that the duration and distribution of anaerobic conditions at this site would be dramatically reduced due to sub-surface drainage tiles that would likely limit prolonged denitrification production, and ultimately N<sub>2</sub>O emissions (Ball et al., 2008). Drain tiles would also encourage the downward movement of N<sub>2</sub>O dissolved in water (Chapter 4), as N<sub>2</sub>O solubility in water increases exponentially with decreasing temperature (Davidson and Firestone, 1988; Heincke and Kaupenjohann, 1999; Roper et al., 2013). Considering the improved drainage conditions at the site and the year-to-year variability among treatments, total N<sub>2</sub>O emissions are relatively low in both years, but not insignificant. The necessity of full season measurements, in addition to multiple years of measurements across varied management scenarios, is highlighted by these results.

### 3.6.2 *Soil properties and N<sub>2</sub>O emissions*

It is unclear what amount of N is released from clover residues when mulched at the soil surface and then recycled back into the rhizosphere versus being lost from the system altogether. However, from soil NO<sub>3</sub><sup>-</sup> measurements, we can see that the removal of above-ground legume residue in the HLF treatment reduced NO<sub>3</sub><sup>-</sup> in bulk soil through the early fall in Year 1 when compared to all other treatments (Fig. 3.2). Early Fall and SP had lower NO<sub>3</sub><sup>-</sup> contents when compared to LF and HLF plots in late Apr., with LF being greater than HLF. This indicates that there is substantial N stored below ground and the timing of incorporation in early Nov. is an effective way to slow N mineralization from incorporated GMr through the winter, but mineralization and nitrification may increase in early spring before cash crop planting, which poses a significant risk for increased environmental N losses.

Increasing NH<sub>4</sub><sup>+</sup> concentrations (net mineralization) in cold soils has been reported (Malhi and Nyborg, 1986; Cookson et al., 2002; Smith et al. 2010), which would be in agreement with our finding in Year 1 (Fig. 3.2). Significantly less NH<sub>4</sub><sup>+</sup> was present in the EF plots when compared to all other treatments at the beginning of Jan.; indicating that the easily decomposed and mineralized substrate was depleted quickly soon after incorporation in Sep. (EF). Immobilization and nitrification has been suggested to be more sensitive in colder (e.g., 2–5°C) soil temperatures (Cookson et al., 2002; Hoyle et al., 2006; Clark et al., 2009), while another study reported little difference in these N dynamics when rye grass was incubated at 3°C

(Korsaeth et al., 2002). Cookson et al. (2002) proposed that heterotrophic organisms may be more competitive for  $\text{NH}_4^+$  than autotrophic nitrifiers in cold soils when sufficient C substrate is present, which would favor immobilization, rather than nitrification. Increased  $\text{NH}_4^+$  concentrations in early Jan. in the SP, HLF, and LF without a parallel increase in  $\text{NO}_3^-$  suggests that mineralization was not limited in cold soils, but nitrification may have been slower at these temperatures, as suggested above.

The pattern of increased  $\text{N}_2\text{O}$  emissions following clover incorporation was mirrored by increased  $\text{CO}_2$  emissions. Microbial and plant respiration are responsible for the production of  $\text{CO}_2$ , therefore, it is not surprising that we measured increased  $\text{CO}_2$  emissions following an increase in substrate availability when clover was incorporated. Cumulative  $\text{CO}_2$  emissions have been used to help explain total  $\text{N}_2\text{O}$  emissions (Snowdon et al., 2013);  $\text{CO}_2$  production is both an indicator of C availability to soil organisms that may be responsible for  $\text{N}_2\text{O}$  production as well as the consumption of soil  $\text{O}_2$ , which creates the conditions needed for denitrification. The increase in substrate availability following GMr incorporation together with an excess of soil water from frequent fall precipitation, as occurred especially in Year 2 (Fig. 3.1), creates ideal conditions for  $\text{N}_2\text{O}$  production through denitrification (Petersen et al., 2013). Moreover,  $\text{N}_2\text{O}$  production would increase when a growing crop is lacking following fall GMr incorporation from greater  $\text{NO}_3^-$  availability (Miller et al., 2008). When a regression of cumulative  $\text{N}_2\text{O}$  (Sep.–June) against cumulative  $\text{CO}_2$  (Sep.–June) was performed on our 2-y data set (data not shown), we do indeed see a weakly positive, yet non-significant relationship ( $R^2 = 0.37$ ;  $p = 0.11$ ;  $n = 8$ ). The relationships of cumulative  $\text{CO}_2$  and  $\text{N}_2\text{O}$  as discussed above are primarily made in studies that have focused on the growing season, as opposed to our study that has concentrated on the off-season. Thus, it is not surprising that our positive relationship with  $\text{CO}_2$  and  $\text{N}_2\text{O}$  is not as strong as those that have been previously documented.

### *3.6.3 Effects of soil freezing and thawing on $\text{N}_2\text{O}$ emissions at spring thaw*

The length, duration, and frequency of freezing and thawing events and the availability of substrate are important drivers of spring thaw  $\text{N}_2\text{O}$  emissions, yet it is difficult to predict which mechanism has the greatest influence. Risk et al. (2014) described spring thaw emissions as having two peaks; the first being from  $\text{N}_2\text{O}$  that had accumulated within the soil profile and released upon soil thawing and the second being produced and emitted at the soil surface (*de*

*novo*), with the latter being larger than the first. Much of the current research suggests that the majority of N<sub>2</sub>O emissions associated with soil freezing and thawing are produced close to the soil surface (Rover et al., 1998; Wagner-Riddle et al., 2010; Maas et al., 2013). However, we would have expected to see more N<sub>2</sub>O emissions earlier in Mar. from *de novo* N<sub>2</sub>O production at the soil surface, as surface soil (0–5 cm) was experiencing complete thawing and then re-freeze on a diel basis. In both years, surface soil temperatures were fluctuating from near freezing up to 10°C on a daily basis for at least a week before the major N<sub>2</sub>O emission event occurred during thaw (Fig. 3.4), while temperatures deeper in the soil profile remained close to 0°C.

The link between soil freezing degree hours ( $\Sigma$ FDH) at 15 cm and N<sub>2</sub>O emissions helped to explain the difference in emissions among treatments in Year 2 of our study, but soil freezing was shallow in Year 1 and no clear relationship with freezing and N<sub>2</sub>O at spring thaw could be made (Fig. 3.3). In contrast to soil freezing being a driver of spring thaw N<sub>2</sub>O emissions, the rate and timing of soil thawing could also have a significant control on these emission patterns. In the first year of study, a rapid increase in air temperature in the second half of Mar., as indicated by 1297  $\Sigma$ TDH (Table 3.4), triggered the complete thawing of the soil profile and the subsequent release of N<sub>2</sub>O at the soil surface. In comparison, the entire month of Mar. had 1266  $\Sigma$ TDH in Year 2, as compared to 1956  $\Sigma$ TDH in Year 1. Soil temperature, and ultimately the depth of freezing, mirrored the fluctuating air temperature and snow level at the surface. A vegetated surface through the winter will trap more snow and provide greater insulation to freezing air temperatures (Williams et al., 2009; Parkin et al., 2013), and is likely why there was greater freezing at the surface in the fall tilled plots, as compared to the vegetated surface of the SP treatment in Year 2 (Fig. 3.3). The frequent freeze–thaw events through the winter in Year 1—as indicated by the fluctuating air temperature in Fig. 3.1 and  $\Sigma$ DH in Table 3.4—likely eliminated the potential for prolonged soil freezing and could be used as an explanation for why treatment differences were not observed in Year 1. This was in contrast to surface and air temperatures in Year 2 that produced two sequential freeze events that allowed the soil to freeze quickly and deeply at the end of Jan. and into Feb. (Fig. 3.2); as indicated by 3532 and 5673  $\Sigma$ FDH in Year 1 and 2, respectively, while  $\Sigma$ TDH was similar between years. Interpreting year to year variation in average air temperature and its subsequent effect on soil freezing and thawing patterns is challenging because of the amount of daily fluctuations. However, quantifying the number of

hours below and above 0°C using  $\Sigma$ DH does provide a clearer picture of the influence of weather on soil processes.

There was a significant difference in total off-season N<sub>2</sub>O emissions from SP clover incorporation as compared to all fall tillage treatments in both years of study, yet in opposite directions in each year, suggesting that weather also has a strong influence on N<sub>2</sub>O emissions. Emissions from SP in Year 1 were 1.9, 3.3, and 8.9 times greater than EF, LF, and HLF during the month of Mar., respectively, which would suggest that either denitrification was greater in SP, or N<sub>2</sub>O was further reduced to N<sub>2</sub> in the fall tillage plots before being emitted at the soil surface. Rather than being tied to freezing characteristics, N<sub>2</sub>O emissions measured from the undisturbed plots (SP) in Year 1 were more likely caused by the rapid increase in air and soil temperature and the availability of substrate directly at the soil surface, which is consistent with other studies (Ball et al., 1999; Ball, 2013). The improved drainage characteristics in the SP plots, as compared to the fall tillage treatments that would have lacked the continuity of soil pores through the profile, may have reduced WFPS slightly that favored the emission of N<sub>2</sub>O, rather than N<sub>2</sub> in Year 1. Improved drainage in SP could also be used as a possible explanation for the low N<sub>2</sub>O emissions in Year 2, when compared to the three treatments with fall tillage that had N<sub>2</sub>O emissions in Mar. that were 5.5, 13.3, and 16.3 times greater than SP in the EF, LF, and HLF treatments, respectively.

Variation in year to year N<sub>2</sub>O emissions across treatments is not surprising when you consider how GMr management systems, such as those in this study, can alter substrate and O<sub>2</sub> supply in soil freezing and thawing conditions from a modification of the bio-physical soil environment system following tillage. Smith et al. (2003) reviewed the interactions of soil physical properties and biological processes and suggested that the production and consumption of N<sub>2</sub>O are biological processes, while the release of N<sub>2</sub>O is a factor of physical soil properties. As evidenced in the temporal pattern of soil  $\Sigma$ FDH at the 15 cm soil depth (Fig. 3.3), the duration and depth of soil ice can be dramatically different from year to year and was a factor of snow depth, which was altered by the presence of crop residue and the timing of fall plowing. The formation of soil ice is highly influenced by soil water content and pore size distribution, which would clearly be modified by fall-tillage, and thus, help to explain why the timing of plowing altered soil-freezing dynamics. Some of the discrepancy in water infiltration from soil freezing and thawing could be from the disruption of soil aggregates (Dagesse, 2013), or macropore

connectivity (Strudley et al., 2008), as freezing and plowing can lead to a decrease in pore size and connectivity (Bullock et al., 1988). A change in the size of soil pores and their connectivity (i.e., tortuosity) between tilled and no-till soils and the concomitant influence on soil freezing and thawing dynamics is an example of how similar soils, even when located in the same field, can have different soil freezing and thawing dynamics (Grant et al., 1990; Parkin et al., 2013).

What was clear from our study was the increase of soil temperature at 15 cm and the subsequent N<sub>2</sub>O emission event; indicating that soil thawing through the soil profile had a control on the timing of the N<sub>2</sub>O burst at spring thaw. The parallel increase in water content and temperature at 15 cm measured in this study was in agreement with Wagner-Riddle et al. (2007) and Mass et al. (2013) who measured peak emissions at spring thaw when soil temperature and water content were increasing together at the soil surface. However, we observed that this relationship was not only occurring at the soil surface, but also at 15 cm, which would suggest that hydrological properties deeper in the soil profile had a more significant control on the timing of N<sub>2</sub>O emissions in our study. In our study, peak N<sub>2</sub>O emissions at spring thaw were timed very closely to complete thawing of the soil profile and the rapid movement of soil water, greater WFPS, and an increase in soil temperature (Fig. 3.4). The timing of N<sub>2</sub>O emissions at spring thaw and the increase in soil water drainage indicates a link between the hydrology at the site and gas exchange at spring thaw, which has been suggested to be a major control on N<sub>2</sub>O emissions (Castellano et al., 2010). Temperature dynamics and the resulting effect on water and gas exchange through the plow depth clearly have a tight control on the timing and magnitude of N<sub>2</sub>O emissions. This could help explain why we measured contrasting emission patterns among GMr treatments during Mar. soil thawing.

As climate change models commonly predict a decrease in winter temperatures and an increase in the frequency of freeze–thaw events (Makoto et al., 2013), snow levels in some temperate northern latitudes will undoubtedly decrease. While a snow reduction could increase soil freezing and subsequent N<sub>2</sub>O emissions, as seen in Year 2 of our study, there is also the possibility for soil freezing to be concentrated at the soil surface with the increase in freeze–thaw events, which could limit N<sub>2</sub>O emissions, as seen in Year 1. A snow removal experiment in a cover-cropped field in New York indicated greater minimum temperatures, but only limited evidence of increased N<sub>2</sub>O emissions from fallow plots that experienced greater soil freezing when snow was removed (Dietzel et al., 2011). Wagner-Riddle et al. (2007) measured a

difference in soil temperatures (5 cm) between bare ( $-3.3^{\circ}\text{C}$ ) and vegetated surfaces ( $+0.1^{\circ}\text{C}$ ) and differences in  $\text{N}_2\text{O}$  emissions (Nov.–Apr.) between treatments were calculated as being 76% higher from bare soil ( $2.9 \text{ kg N}_2\text{O ha}^{-1}$ ) as compared to those measured from a vegetated surface ( $0.7 \text{ kg N}_2\text{O ha}^{-1}$ ). However, as indicated in our study, reduced snow levels can be as a result of frequent freeze–thaw events that reduce prolonged and deep soil freezing and ultimately the number of soil freezing days (Henry, 2008). Some of the predictions of increased soil freezing with a reduced snow pack are a result of snow removal experiments, which may not be a realistic simulation of actual soil freezing dynamics as a result of weather that causes a reduction in snow levels.

### 3.7 Conclusions

It is clear from this two-year study that there is potential for considerable  $\text{N}_2\text{O}$  emissions from perennial legume GMr crops, regardless of the timing or season of incorporation. Delaying GMr incorporation until spring did reduce  $\text{N}_2\text{O}$  emissions in one of our study years, but also produced the highest emissions at spring thaw in the other year. Early Fall GMr incorporation led to greater emissions in the fall, while delaying GMr incorporation until Nov. (i.e., LF, HLF) only proved to shift the season of emissions until after the winter. The removal of GMr biomass in the HLF treatment did not reduce overall  $\text{N}_2\text{O}$  emissions, which followed a similar temporal emission pattern as LF; indicating that there is considerable N located belowground in a 2-yr old legume GMr crop that becomes susceptible to off-season N losses as  $\text{N}_2\text{O}$ . The use of aboveground legume biomass for forage may be a more efficient use of N, as there was no significant increase in  $\text{N}_2\text{O}$  emissions when this biomass was soil incorporated. However, it should be noted that there was also an increase in WFPS during thaw in Year in HLF plots, which may have led to the consumption of  $\text{N}_2\text{O}$ , rather than a decrease in production.

The lack of a clear relationship with the length and duration of soil freezing, as measured by  $\Sigma\text{FDH}$ , and the emission of  $\text{N}_2\text{O}$  at spring thaw was surprising. However, when air temperature was interpreted using both cumulative freezing and thawing hours this information was helpful in interpreting seasonal differences between years, which were used in interpreting gas emissions at spring thaw. The hydrological influence on spring thaw emissions were evident in both years of study and emphasized the importance of understanding the belowground

dynamics of soil freezing, gas accumulation, and subsequent soil thawing and gas emissions from the soil surface.



## **4. WINTER SOIL PROFILE N<sub>2</sub>O CONCENTRATIONS AND DISSOLVED N<sub>2</sub>O IN TILE DRAINAGE WATER WITH VARIED TIMING OF RED CLOVER TILLAGE**

### **4.1 Preface**

The goal of Chapter 3 was to analyze off-season (Sep.-June) surface N<sub>2</sub>O emissions as affected by the timing of red clover GMr tillage and weather over two years. The focus in Chapter 4 is on belowground dynamics of N<sub>2</sub>O and CO<sub>2</sub> during those emission events. The physical release of N<sub>2</sub>O stored below a frozen soil layer is one mechanism that contributes to elevated N<sub>2</sub>O emissions at spring thaw. Much of the previous work in this area has focused on N<sub>2</sub>O diffusion to the atmosphere, with little discussion of the possibility of its downward movement in the soil profile with water. In Atlantic Canada, the installation of sub-surface drainage improves soil conditions and extends the limited growing season—especially in the spring when snowmelt and high precipitation can make field accessibility impossible. However, discharging soil water that is supersaturated with N<sub>2</sub>O is a pathway for N<sub>2</sub>O emissions that may be overlooked. Dissolved N<sub>2</sub>O in drainage water was collected from plots that included all phases of the 4-yr crop rotation throughout the study period.

## 4.2 Abstract

Emissions of  $\text{N}_2\text{O}$  at spring thaw are caused by the rapid production of  $\text{N}_2\text{O}$  and the physical release of previously produced  $\text{N}_2\text{O}$  under frozen soil as thawing begins, yet the downward movement and the potential for further emissions to the atmosphere at sites with drainage tiles installed has rarely been considered. Concentrations of  $\text{N}_2\text{O}$  within the soil profile (7.5–57.5 cm) from Dec. to May over two years were examined in an experiment with varied season and timing of GMr incorporation to examine how the temporal dynamics of belowground  $\text{N}_2\text{O}$  relate to  $\text{N}_2\text{O}$  emissions at the soil surface and  $\text{N}_2\text{O}$  dissolved in drainage water. Treatments included: GMr incorporated in Sep. (Early Fall; EF), Nov. GMr incorporation with aboveground biomass removed during the growing season (Hayed Late Fall; HLF), GMr incorporated in Nov. (Late Fall; LF), and undisturbed GMr that was incorporated in May (SP). In Year 1, Soil  $\text{N}_2\text{O}$  concentrations reached a maximum at the 15 and 30 cm depths in the SP and EF treatments on 8 Mar. 2012 with a mean ( $\pm$ SD) of  $120 \pm 25 \mu\text{L L}^{-1}$ , while the highest concentrations at 50 cm were measured on 11 Mar. 2012 from EF ( $109 \mu\text{L L}^{-1}$ ). During spring thaw in Year 2, SP produced the lowest  $\text{N}_2\text{O}$  concentrations at all depths, while all other treatments had a mean of  $98 \pm 20 \mu\text{L L}^{-1}$  (15 cm) and  $113 \pm 37 \mu\text{L L}^{-1}$  at 30 to 50 cm. Following extensive drainage, soil concentrations at all depths declined to background levels ( $0.3 \mu\text{L L}^{-1}$ ), but the decline was more rapid in Year 2 due to a 54% increase in drainage water. There was no difference in dissolved  $\text{N}_2\text{O}$  ( $\mu\text{g N}_2\text{O-N L}^{-1}$ ) in tile drain flow among treatments in Year 1 or 2 ( $p = 0.7\text{--}0.97$ ), yet increased during spring thaw in all treatments with maximum values of 167 to 269  $\mu\text{g N}_2\text{O-N L}^{-1}$ . Overall losses of dissolved  $\text{N}_2\text{O}$  ( $\text{g N}_2\text{O-N ha}^{-1}$ ) were 14 and 18% of  $\text{N}_2\text{O}$  emitted from the soil surface during the same period and are therefore a significant pathway for  $\text{N}_2\text{O}$  losses.

## 4.3 Introduction

Emissions of  $\text{N}_2\text{O}$  from the agricultural sector are a substantial component of global  $\text{N}_2\text{O}$  emissions (Janzen, 1998; Butterbach-Bahl et al., 2004; Ambus et al., 2011), yet despite decades of research devoted to the processes that contribute to these emissions there is less of an understanding on the effects of cropping practices on off-season emissions in temperate climates when compared to in-season emissions. Canadian off-season  $\text{N}_2\text{O}$  emissions were estimated to

be 30% of total annual emissions from cropland (Smith et al., 2004), although a much greater proportion of emissions have been reported in eastern Canada (i.e., 30–90%; Wagner-Riddle et al., 2007), as well as emissions from northern Europe (e.g., Rover et al., 1998; Syvasalo et al., 2004). Off-season emissions are typically associated with the actions of freezing and thawing soils, as such, the majority of these emissions occur as brief, yet vigorous events during spring thaw (Christensen and Tiedje, 1990; van Bochove et al., 2000b; Jacinthe et al., 2002; Wagner-Riddle et al., 2007).

Because soil surface N<sub>2</sub>O emissions are a result of the complex interactions of soil biological, physical, and chemical properties within the soil profile and at the soil surface (Smith et al., 2003; Castellano et al., 2010; Ball et al., 2013), it can be difficult to pinpoint how management decisions affect soil processes responsible for these emissions. The season and timing of GMr incorporation has not been thoroughly evaluated in the field with respect to freeze–thaw related N<sub>2</sub>O emissions, however a legume forage pasture in Germany was shown to have multiple daily N<sub>2</sub>O flux rates of 50 to 100 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> that were associated with soil freeze–thaw cycles, as well as events that followed legume incorporation at the end of the three year forage cycle (Kaiser et al., 1998). When soybean residues were incorporated in a laboratory experiment, N<sub>2</sub>O emissions during 4-d incubation at 1°C had a four-fold increase in a silty clay soil when compared to a sandy loam, which was only one example of the intricate interactions with soil type, crop residue addition, and the length and magnitude of freezing on the dynamics of N<sub>2</sub>O emissions when spring thaw conditions were simulated (Pelster et al., 2013). Denitrification may be limited in coarse textured soils by C availability when organic matter additions are low, but in cropping systems using GMr, the availability of C and N substrates is likely not a factor.

Most of the previous work on overwinter N<sub>2</sub>O emissions from Canadian agricultural soils has demonstrated that the majority of N<sub>2</sub>O emissions are a result of denitrification at the soil surface (Furon et al., 2008; Wagner-Riddle et al., 2008; Kariyapperuma et al., 2011). However, another mechanism that contributes to high N<sub>2</sub>O emission rates at spring thaw is the physical release of trapped N<sub>2</sub>O under a frozen soil layer (Goodroad and Keeney, 1984; Risk et al., 2013). In soils that experience freezing and thawing, water potential and temperature gradients have a significant control on the production, consumption, and location of N<sub>2</sub>O within the soil profile (Clough et al., 2005). Therefore, there is a need to understand how soil conditions and cropping

patterns influence the production and movement of N<sub>2</sub>O within the soil profile. For a more complete understanding of the soil processes involved in surface gas flux, concentrations of CO<sub>2</sub> and N<sub>2</sub>O gas from the soil profile have been studied in a variety of different agricultural (Burton and Beauchamp, 1994; Wagner-Riddle et al., 2008; Yanai et al., 2011; Risk et al., 2014) and forest ecosystems (Billings et al., 1998; Risk et al., 2002; Goldberg et al., 2010; Lavoie et al., 2013), in addition to work done in the Canadian Arctic (Brummell et al., 2012; 2014). Risk et al. (2014) reported quantitative evidence of spring thaw related N<sub>2</sub>O emissions from the surface being comprised of an initial physical release of N<sub>2</sub>O that had accumulated under a frozen soil layer, and a second surface N<sub>2</sub>O emission event that was predominately due to N<sub>2</sub>O production at the soil surface.

In cold climates, the location, temperature, and amount of soil water has the ability to greatly modify or regulate processes and properties controlling soil gas concentrations and the proportion of N<sub>2</sub>O that dissolves in soil water (Heincke and Kaupenjohann, 1999). However, soil freezing will limit water drainage through the soil profile (Kojima et al., 2013) as well as the diffusion of gases out of the soil profile (van Bochove et al., 2001a). Soil ice near the surface may restrict water and gas exchange during winter and allow N<sub>2</sub>O to accumulate within the soil profile (Burton and Beauchamp, 1994). As soil ice turns to liquid, supersaturated N<sub>2</sub>O in drainage water may contribute to surface N<sub>2</sub>O if a pathway for gas exchange with the atmosphere is present (Yanai et al., 2011; Risk et al., 2014) or is transported out of the upper soil profile as drainage water (Burton et al., 2012). Therefore, field management decisions that modify soil water contents and hydrological processes through a change in soil pore size and distribution may also affect N<sub>2</sub>O production and release to the atmosphere.

Because N<sub>2</sub>O dissolves in water at exponentially higher rates as water temperature drops closer to 0°C (Heincke and Kaupenjohann, 1999) the downward movement of N<sub>2</sub>O at spring thaw needs to be considered. The first published report of dissolved N<sub>2</sub>O in drainage water was by Dowdell et al. (1979) from tile-drained agricultural fields in South East England. Although this study was published over 35 years ago, this N loss pathway is still not very well understood, and typically not considered in overall N loss budgets. When the amount of N<sub>2</sub>O that is stored or newly produced within the soil profile at spring thaw is considered in combination with the amount of water that moves through the soil profile at this time, fields that have improved drainage are potentially emitting a significant amount of N<sub>2</sub>O at the drain outlet or drainage

ditches. The effect of a soil's water regime on the concentration and location of dissolved  $\text{N}_2\text{O}$  within a soil profile was described by Heincke and Kaupenjohann (1999) as follows: (i) liquid or solid water acts as a diffusion barrier in soil for  $\text{N}_2\text{O}$  emissions because gas diffusion in air is  $10^4$  times greater than diffusion in water (Fang and Moncrieff, 1999); (ii) cold soil water acts as a store of  $\text{N}_2\text{O}$ , as  $\text{N}_2\text{O}$  solubility is inversely related to water temperature; (iii) water becomes a rapid mode for  $\text{N}_2\text{O}$  transport through the soil profile when temperature and pressure gradients occur with soil depth.

Agricultural systems that include green manure (GMr) in their crop rotation may emit substantial  $\text{N}_2\text{O}$  emissions that occur outside the growing season, especially in climates that experience extended periods of soil freezing and thawing. Concentrations of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  over time and at different soil depths along with details on some soil physical properties provides critical information towards a better understanding of the mechanisms involved during these elevated emissions. The objective of this study was to determine the effects of incorporated red clover on the dynamics of soil profile (7.5–57.5 cm)  $\text{N}_2\text{O}$  concentrations during two soil freezing and thawing seasons (Dec. to Apr.) in Truro, NS. Characterizing and quantifying  $\text{N}_2\text{O}$  losses in water collected from sub-surface drainage tiles and whether they coincide with changes in  $\text{N}_2\text{O}$  content in the soil profile of GMr plots is an additional objective of this study.

## 4.4 MATERIALS AND METHODS

### 4.4.1 Field site description and experimental design

All field data were collected at the Brookside experimental site for organic agriculture at Dalhousie University's Agricultural Campus, Truro, Nova Scotia, Canada (45°23'24.72" N lat; -63° 15'16.15" W long). Field data were collected from Dec. to Apr. in Year 1 (2011-12) and 2 (2012-13). Soil and climate at the Brookside experimental site are described in Chapter 3. This study was part of a larger field experiment that included a red clover (*Trifolium pratense* L.) – spring wheat (*Triticum aestivum* L.) crop sequence within a 4-yr rotation (i.e., soybeans–red clover–red clover–spring wheat). The overall experiment was designed in a randomized block design with four main plots (16 x 75 m) and four sub-plots (16 x 16 m) representing all four phases of the crop rotation in each main plot. Each treatment and phase was then replicated over three blocks. The 2<sup>nd</sup> year of clover (i.e., Year 3 phase of the rotation) was the focus of this study. Treatments differed in management and timing of GMr incorporation using a moldboard plow (Table 4.1). Treatments included: (1) Clover incorporated in Sep. (Early Fall; EF), (2) Clover incorporated in Nov., with aboveground clover biomass removed (Hayed Late Fall; HLF), (3) Clover incorporated in Nov. (Late Fall; LF), and (4) Clover undisturbed until incorporation in May (Spring; SP). A full description of treatments is found in Table 4.1 and the plot layout, including the placement of drainage tiles and gas sampling locations, is included in Appendix 1.

**Table 4.1.** Description and timing of GMr treatments at the experimental site 2011–2013.

| Treatments                        | Activity   | Year 1       | Year 2       |
|-----------------------------------|--|--------------|--------------|
| Hayed Late Fall                   | Clover biomass harvested as hay                      | 23 June 2011 | 18 June 2012 |
| Early Fall, Late Fall, and Spring | Clipped clover, residue remained on the soil surface | 23 June 2011 | 18 June 2012 |
| Hayed Late Fall                   | Clover biomass harvested as hay                      | 18 Aug. 2011 | 31 July 2012 |
| Early Fall, Late Fall, and Spring | Clipped clover, residue remained on the soil surface | 18 Aug. 2011 | 31 July 2012 |
| Early Fall                        | Red clover incorporation                             | 17 Sep. 2011 | 18 Sep. 2012 |
| Hayed Late Fall and Late Fall     | Red clover incorporation                             | 2 Nov. 2011  | 7 Nov. 2012  |
| Spring                            | Red clover incorporation                             | 2 May 2012   | 1 May 2013   |

#### 4.4.2 *Climate and soil temperature and moisture monitoring*

Soil temperature and volumetric water content were measured in each plot ( $n=12$ ) using a single 5TE sensor (Decagon Devices, Pullman, WA, USA) at soil depths of 15, 30, and 50 cm. Hourly soil temperatures were logged to an EM-50 data logger (Decagon Devices, Pullman, WA, USA). Sensors were installed by excavating a narrow hole with a tree planter's shovel in each plot, and then the sensor is inserted perpendicular to the surface in the undisturbed sidewall of the narrow hole. Each sensor had a soil volume of influence of 715 cm<sup>3</sup>. Air temperature and precipitation were measured on-site using a shielded thermocouple and tipping bucket with hourly measurements logged to a CR200X data logger (Campbell Scientific, Logan, UT, USA).

#### 4.4.3 *Drainage water*

Sub-surface drainage water was collected through a tile drain system installed to a depth of 0.8 m. Each plot (16 x 75 m) consists of two tile lines placed 6.5 m apart that combined into one line at end of the plot, which drained an area that included all four phases of the rotation. Each line runs into an on-site building with tipping buckets to measure drainage flow (m<sup>3</sup> ha<sup>-1</sup>). Buffer zones between plots are tile drained as well, hydrologically isolating each plot. Soil water infiltration becomes restricted with soil freezing (Kojima et al., 2013); therefore, periods of limited drainage become an indicator of soil freezing through the soil profile and a rapid increase in drainage indicates soil thawing.

#### 4.4.4 Soil profile N<sub>2</sub>O

The concentration and mass of N<sub>2</sub>O within the soil profile (7.5–57.5 cm) was evaluated via soil atmosphere sampling tubes, as described by Burton and Beauchamp (1994), Jacinthe and Dick (1996), and Goldberg et al. (2008). Each plot ( $n=12$ ) had two probes installed down the center, placed 5 m apart by auguring a hole slightly larger than the probe diameter. Probes were installed at least two weeks before the first measurements were made. Soil gas measurements were made from three depths (i.e., 15, 30, 50 cm). Soil atmosphere sampling tubes were installed in 2<sup>nd</sup> year red clover plots, and were sampled on the same bi-weekly schedule as surface flux measurements beginning in Dec. 2011 until Apr. 2012 (Year 1), and again in Year 2 (Dec. 2012–May 2013).

Total N<sub>2</sub>O mass (mg N<sub>2</sub>O-N m<sup>-2</sup>) in each depth (gaseous and aqueous) was calculated using N<sub>2</sub>O  $\mu\text{L L}^{-1}$  concentrations from each depth and the proportion of the soil pore space that is filled with air (gaseous N<sub>2</sub>O) and water (aqueous N<sub>2</sub>O). Bulk density measurements from each plot were used to determine the total pore space and measurements of soil water content (vol.) and soil temperature, as described above, were used to determine the proportion of the pore space filled with water or ice.

Calculation of N<sub>2</sub>O content in each depth ( $M_{\text{gas}}$ ; mg N<sub>2</sub>O-N m<sup>-3</sup>) was made using;

$$M_{\text{gas}} = [\text{N}_2\text{O}_{\text{gas}}] V_{\text{gas}} \quad [1]$$

where  $[\text{N}_2\text{O}_{\text{gas}}]$  is N<sub>2</sub>O concentration (mg N<sub>2</sub>O-N m<sup>-3</sup>),  $V_{\text{gas}}$  is the air-filled porosity (m<sup>3</sup> air m<sup>-3</sup> soil) estimated in each soil depth using bulk density, and water content at the time of sampling. Hourly soil temperature measurements together with the liquid water content were used to determine the proportion of the soil pore space occupied by soil ice.

The mass of N<sub>2</sub>O was determined for the specific volume of air within each soil volume (0.15 m<sup>3</sup>) at each sampling depth that had midpoints at 15- (7.5–22.5 cm), 30- (22.5–37.5 cm), and 50-cm (42.5–57.5 cm) and assuming a 1-m<sup>2</sup> soil area. Two bulk density measurements were made in each plot in Nov. of Year 1 and 2 from an intact soil core (0–100 cm) that was divided into four sections (0–15, 15–30, 30–60, and 60–100 cm). Linear interpolation was then used to determine the bulk density for the soil volume associated with gas measurements. The mass of N<sub>2</sub>O dissolved in soil water before and during freezing was calculated using Henry's Law as described by Risk et al. (2014).



Henry's law, modified for closed systems, was used to calculate aqueous N<sub>2</sub>O content;

$$M_{\text{aq}} = M_{\text{gas}} \beta V_{\text{aq}}/V_{\text{gas}} \quad [2]$$

where  $M_{\text{aq}}$  is the mass of dissolved N<sub>2</sub>O present in each layer (mg m<sup>-3</sup>),  $\beta$  is the Bunsen absorption coefficient (dimensionless), and  $V_{\text{aq}}$  is the volumetric liquid water content (m<sup>3</sup> H<sub>2</sub>O m<sup>-3</sup> soil). The Bunsen coefficient used in each calculation was determined as a function of soil temperature at the time of sampling.

#### 4.4.5 Dissolved N<sub>2</sub>O in drainage water

Dissolved N<sub>2</sub>O in tile drainage water was measured by determining the concentration of N<sub>2</sub>O in the pressurized headspace of a sealed glass vial containing water from drainage tiles. During periods of high flow, water samples (4 mL) were taken directly from a spigot at the end of the tile line using a gas-tight syringe and injected into 12 mL glass vials. Prior to sampling, a mercuric chloride (HgCl<sub>2</sub>) solution (50 µL of 0.02 M) was added to the glass vial to eliminate bacterial activity and the possibility of further gas evolution within the water sample (Roper et al., 2013). A headspace to water ratio of 3:1 has been identified as the optimal ratio for accurate and reproducible results of dissolved gases in water (Jahangir et al., 2012). Background concentrations of dissolved N<sub>2</sub>O are assumed to be around 0.25–0.35 µg N<sub>2</sub>O-N L<sup>-1</sup> at 20°C when equilibrium with the atmosphere is reached (Reay et al., 2004a; Minamikawa et al., 2013). Gas analysis took place in Truro, NS, as described in Chapter 3. Aqueous and N<sub>2</sub>O gas phase equilibrium is controlled by water temperature and pressure in addition to the solubility of N<sub>2</sub>O dissolved in water, which was calculated using a modified method of Weiss and Price (1980) as described by Roper et al. (2013). Corrections to calculated N<sub>2</sub>O solubility coefficients for salinity (EC), as described in Weiss and Price (1980), were not necessary for the expected range of drainage water salinity in this study.

#### 4.4.6 Statistical analysis

Soil profile N<sub>2</sub>O and CO<sub>2</sub> concentrations were evaluated for the effects of Treatment and Depth over time evaluated using a doubly repeated measures mixed model analysis following log<sub>(10)</sub> transformation. Repeated factors were soil Depth and Time for each year and random effects were Block and Block x Treatment. Differences among treatments in N<sub>2</sub>O mass within

the soil profile (7.5–57.5 cm) were subjected to a repeated mixed model, which used Block and Block x Treatment interaction term as random factors. Winter (Dec.–Apr.) N<sub>2</sub>O emissions and dissolved N<sub>2</sub>O in drainage water were evaluated for treatment differences using a repeated measures mixed model that treated Block as the random effect. Treatment means were separated with Tukey's post-hoc test and when a significant interaction was present, means were separated with a test of the simple main effects (slices). Pearson's correlation coefficients were determined to evaluate linear relationships of CO<sub>2</sub> and N<sub>2</sub>O using data collected on 12 days from Jan. to Apr. that dissolved N<sub>2</sub>O and the soil atmosphere were sampled together over the two year study. All effects were considered significant when  $p \leq 0.05$ , and statistical tests were performed using JMP software (SAS Institute Inc. Cary, NC, USA).

## 4.5 RESULTS

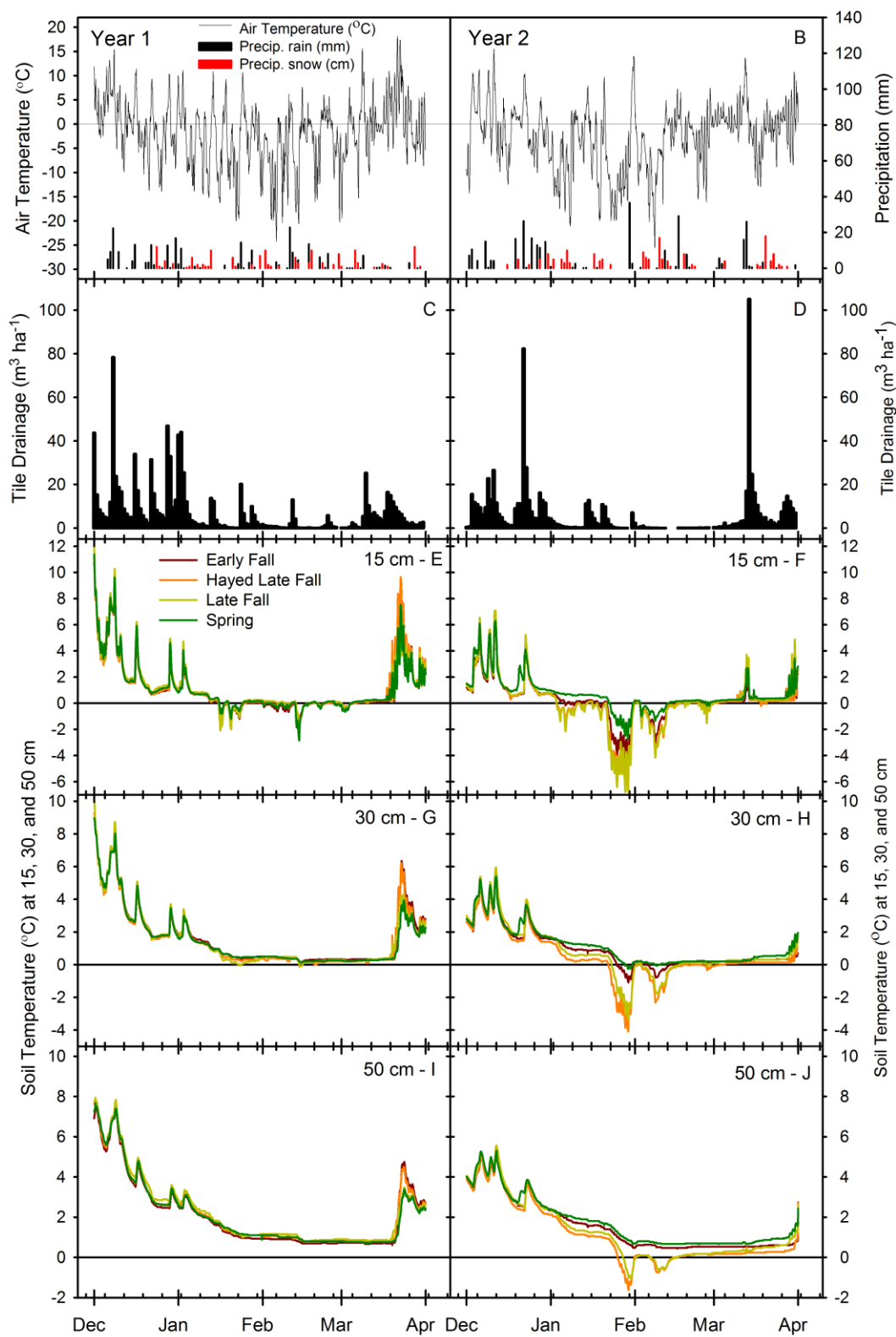
### 4.5.1 *Weather, tile drainage, and soil temperature*

Air temperatures between years were not different in Feb. or Mar., but were warmer through Dec. in Year 1, and twice as cold through Jan. in Year 2 (Fig. 4.1). Further details on temperature dynamics between years are given in Chapter 3. Total precipitation (rain and snow) in Dec. was similar in Year 1 and 2, while rainfall diminished greatly in Jan. and there were similar snowfalls of 45 and 40 cm, respectively. There was nearly twice as much rain in Feb. of Year 1 as compared to Year 2 (17 mL). Frequent freezing and thawing cycles were recorded at the beginning of Mar. (Fig. 4.1 A, B) as air temperature began to fluctuate  $\pm 10^{\circ}\text{C}$  around the  $0^{\circ}\text{C}$  mark that initiated a soil thaw. The amount and timing of precipitation during spring thaw had a significant control on the rate of soil thawing. Specifically, during the month of Mar. of Year 1, there was only 15 mm of rain, as compared to 108 mm in Year 2, but snowfall levels during this month were similar, with 47 and 40 cm in Year 1 and 2, respectively. Drainage at spring thaw was nearly five times greater in Year 2, as compared to Year 1 (Fig. 4.1 C, D), which was likely influenced by heavy precipitation events that began in the second week of Mar. as well as greater soil freezing that greatly reduced water drainage prior to the thaw (Chapter 3). This was in contrast to Year 1, when there were very few precipitation events during the month of Mar. and drainage was greater during the period Dec.–Mar. The average water drainage volume from Dec. to the end of Mar. was  $948 \text{ m}^3 \text{ H}_2\text{O ha}^{-1}$  in Year 1, as compared to  $756 \text{ m}^3 \text{ H}_2\text{O ha}^{-1}$  in Year 2 when considered across all treatments. The proportion of total drainage for the study period was similar in Dec. between years (i.e., ca. 51%). During Jan., 24 and 12% of the total drainage occurred in Year 1 and 2, respectively. There was minimal drainage through Feb., but 18 and 38% of the total drainage occurred in Mar. of Year 1 and 2, respectively.

The pattern and extent of soil freezing is closely linked to the absence of snow cover and a prolonged period of air temperatures  $<0^{\circ}\text{C}$  (Fig. 4.1 E–J). In Year 1, freezing temperatures were immediately followed by temperatures  $>0^{\circ}\text{C}$  and this pattern continued for most of the

winter. As a result, soil freezing was minimal in all treatments, as indicated by the increased frequency of water drainage that occurred when soil temperatures were above 0°C. Beginning in mid-Mar., soil temperatures rose quickly in response to a rapid increase in air temperature.

In Year 2, there was an initial period of soil freezing that penetrated to 50 cm and lasted for 1-wk at the end of Jan. (Fig. 4.1). Soil temperatures at all depths quickly rose to 0°C when air temperatures went from -10 to +10°C in a period of 24 h during the first week of Feb. Following this period, air temperatures again remained below 0°C until mid-Feb. when they began to fluctuate above and below 0°C. At the same time, there were periods of rain without any water drainage; an indication that ice had likely formed at the soil surface. What was common between years was the prolonged period that soil temperatures hovered at or very close to 0°C. As discussed in detail in Chapter 3, the Nov. tillage treatments had greater soil freezing than the undisturbed treatment (SP) in Year 2, but no clear differences were observed in Year 1. This effect could be seen at all soil depths in Year 2, but minimal soil freezing at depth in Year 1 was likely why this effect was not observed.

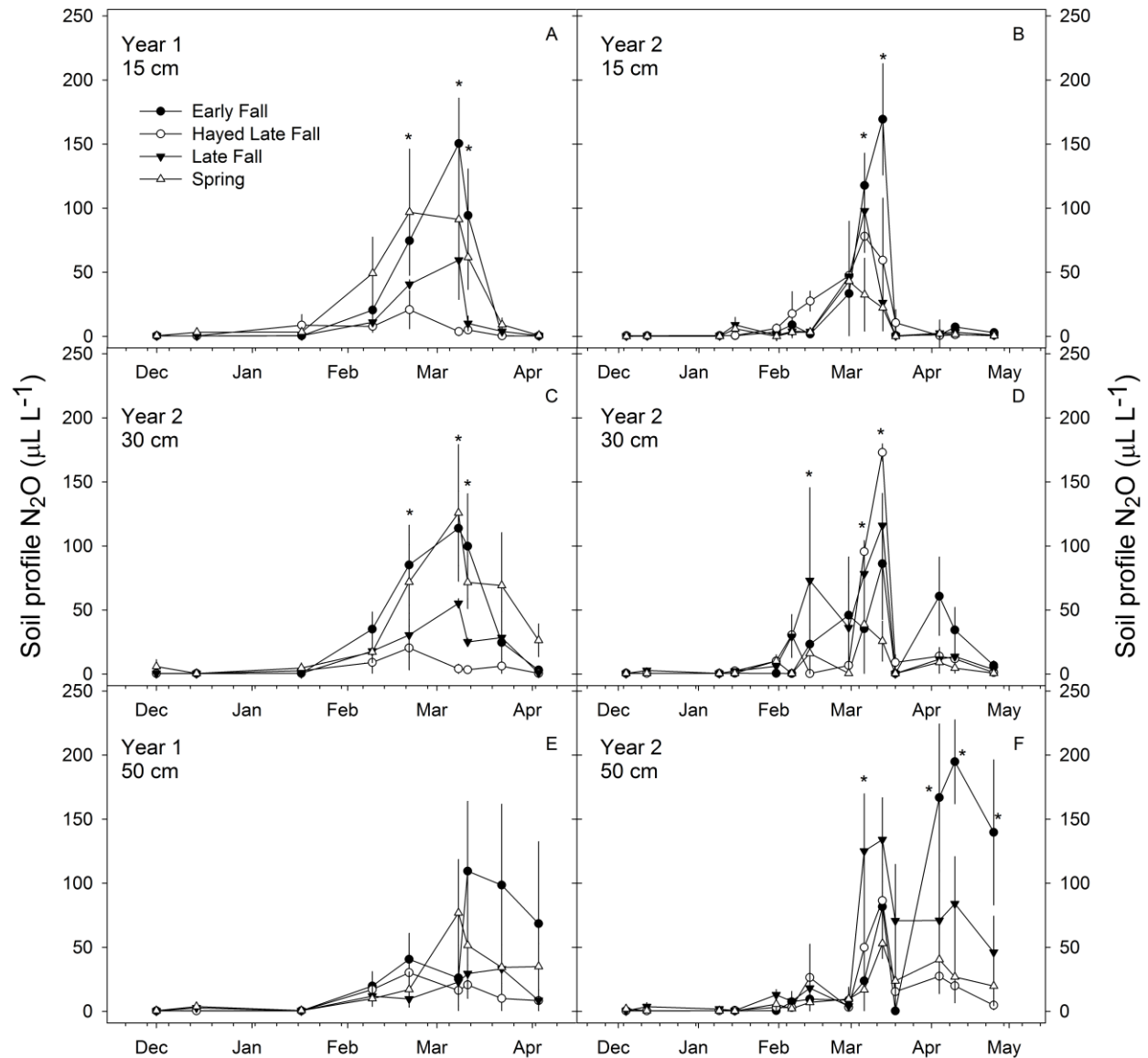


**Fig. 4.1.** Air temperature and precipitation (snow + rain) measured at the experimental site from Dec. to Apr. in Year 1 (A) and 2 (B). Average drainage water from tile lines ( $n=12$ ) is shown in panels C and D. Soil temperatures ( $n=3$ ) for clover incorporation treatments from the 15 (E, F), 30 (G, H), and 50 (I, J) cm soil depths.

#### 4.5.2 Soil N<sub>2</sub>O concentration

In Year 1, the effect of treatment was significant ( $p=0.04$ ) when considered across all sample dates and depths (Fig. 4.2). Early Fall and SP tillage treatments had an overall mean N<sub>2</sub>O concentration of 36.3  $\mu\text{L L}^{-1}$ , which was much greater than HLF (7.7  $\mu\text{L L}^{-1}$ ) while LF was intermediate (15.7  $\mu\text{L L}^{-1}$ ). The lowest ( $p<0.001$ ) N<sub>2</sub>O concentrations across all depths and treatments were measured from 1 Dec. 2011 to 17 Jan. 2012 (1.31  $\mu\text{L L}^{-1}$ ), while the highest were on 8 Mar. 2012 (65.6  $\mu\text{L L}^{-1}$ ). Concentrations declined by 64% on 3 Apr. 2012. There were no differences ( $p=0.16$ ) in N<sub>2</sub>O concentrations among soil depths, nor was there an interaction of Treatment x Depth ( $p=0.24$ ). There was a Time x Depth interaction ( $p<0.001$ ) in Year 1 as a result of higher N<sub>2</sub>O concentrations measured at lower depths near the end of the study period (22 Mar. 2012 to 3 Apr. 2012).

There were no overall differences ( $p=0.14$ ) in soil profile N<sub>2</sub>O concentrations among treatments in Year 2 (Fig. 4.2), despite a two-fold increase in EF, HLF, and LF treatments as compared to SP (10.6  $\mu\text{L L}^{-1}$ ). The highest N<sub>2</sub>O concentrations across all treatments and depths were measured at the height of spring thaw (88.4  $\mu\text{L L}^{-1}$ ; 13 Mar. 2013); these were quickly followed by the lowest only five days later (18 Mar. 2013), representing an overall change in concentration of 76.88  $\mu\text{L L}^{-1}$ . The Treatment x Time interaction was highly significant ( $p<0.001$ ) because N<sub>2</sub>O concentrations across all soil depths were lowest in the SP tillage treatment on 6<sup>th</sup> and 13<sup>th</sup> Mar. 2013. This was followed by the highest concentrations being measured from EF plots that were 1.7, 2.2, and 2.4 times greater than all other treatments on the 4<sup>th</sup>, 10<sup>th</sup>, and 25<sup>th</sup> of Apr. The highest concentrations across soil depths ( $p<0.001$ ) were measured from the 50 cm depth, which were 48% greater than the mean of 18.29  $\mu\text{L L}^{-1}$  over the 15 and 30 cm soil depths. Similar to Year 1, the Depth x Time interaction was significant ( $p<0.001$ ), when surface (15 cm) N<sub>2</sub>O concentrations were greater than the 30 cm soil depth from 28 Feb. to 6 Mar. 2013. Also similar to Year 1, on 18 Mar. 2013, N<sub>2</sub>O concentrations became higher at the lowest soil horizon, which was a trend that continued until the end of the study period on 25 Apr. 2013.



**Fig. 4.2.** Soil profile  $N_2O$  concentrations at 15 (A, B), 30 (C, D), 50 (E, F) cm from Dec. to Apr. in Year 1 and Dec. to May in Year 2. Following a significant Treatment  $\times$  Time interaction ( $p \leq 0.05$ ) differences among treatments among sample dates are indicated with an asterisk. Error bars represent the SEM ( $n=3$ ).

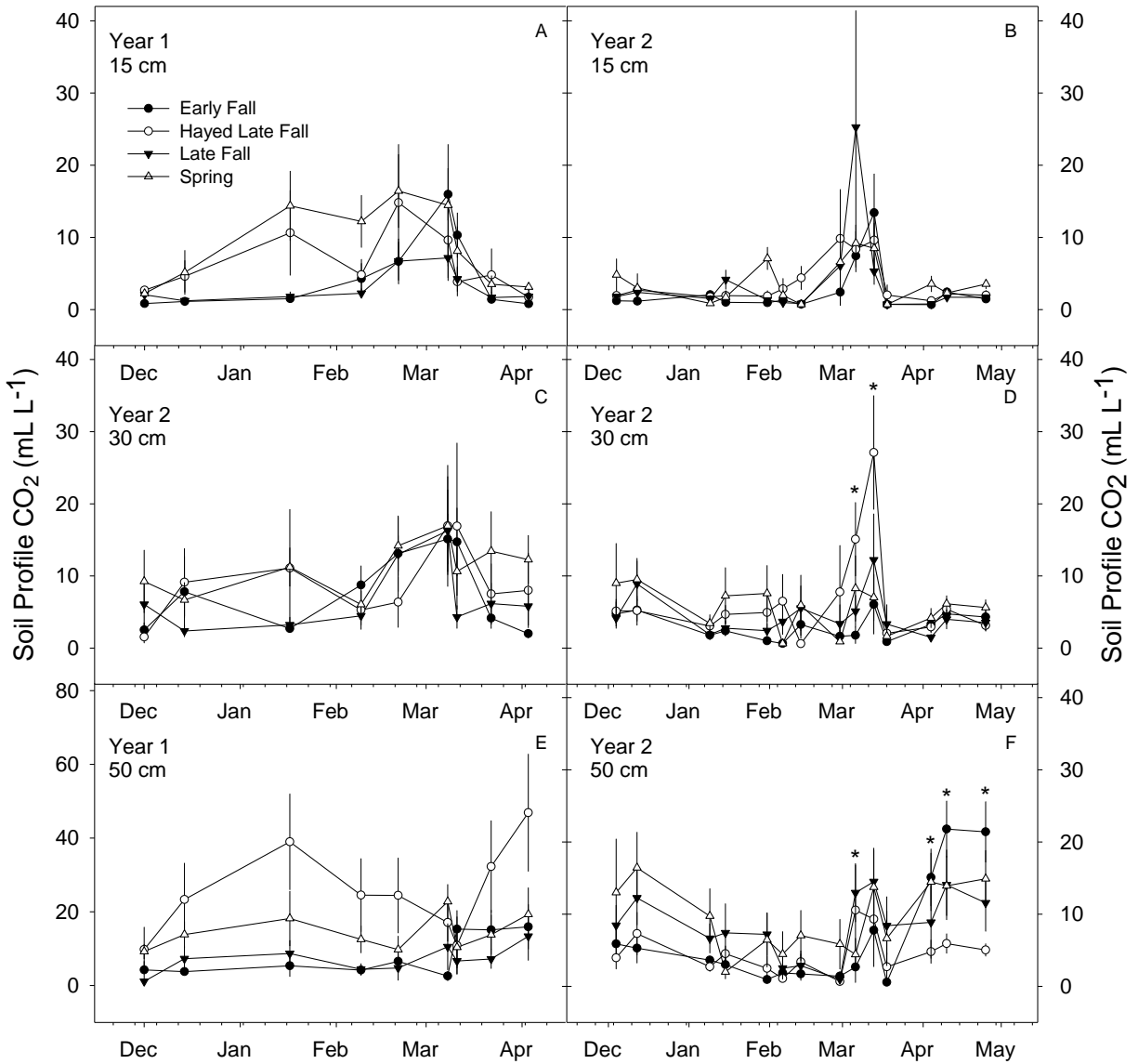
#### 4.5.3 Soil $CO_2$ concentration

In Year 1, the HLF and SP treatments had the highest  $CO_2$  concentrations ( $12.2 \text{ mL L}^{-1}$ ) across all soil depths and sampling dates ( $p = 0.03$ ); these were twice as great as EF and LF ( $6.2 \text{ mL L}^{-1}$ ) treatments (Fig. 4.3). Across all depths and treatments, the highest  $CO_2$  concentrations were measured on 8 Mar. 2012 ( $13.2 \text{ mL L}^{-1}$ ), as compared to the lowest  $CO_2$  concentrations that were measured on the first sample date in Dec. ( $4.3 \text{ mL L}^{-1}$ ). Overall, the greatest ( $p < 0.001$ )  $CO_2$

concentrations were measured at 50 cm ( $13.2 \text{ mL L}^{-1}$ ), which was 56% larger than the mean at 30 cm as compared to the smallest concentrations measured at 15 cm ( $5.82 \text{ mL L}^{-1}$ ). A significant Treatment interaction with Depth ( $p < 0.001$ ) was due to the HLF treatment being different than all other treatments ( $p = 0.02$ ) at 50 cm. The Time x Treatment interaction was not significant ( $p = 0.08$ ), but Time x Depth was ( $p < 0.001$ ). The highest ( $p < 0.001$ ) concentrations at 15 cm across treatment were measured between 21 Feb. and 8 Mar. 2012 ( $11.52 \text{ mL L}^{-1}$ ), but were reduced by 42% three days later. At 30 cm, the highest concentrations ( $p = 0.01$ ) were measured from 21 Feb. to 11 Mar., until a 51% reduction occurred on 22 Mar. 2012. At 50 cm, the highest ( $p < 0.001$ ) of all concentrations within the soil profile were measured on the last sampling date (4 Apr. 2012), with an average of  $22.2 \text{ mL L}^{-1}$  across all treatments.

In Year 2 (Fig. 4.3), there was no overall effect of Treatment ( $p = 0.46$ ), but the highest ( $p < 0.001$ )  $\text{CO}_2$  concentrations during the study period were measured between 6 Mar. and 13 Mar. 2013 ( $10.2 \text{ mL L}^{-1}$ ). Five days later one of the smallest concentrations ( $2.5 \text{ mL L}^{-1}$ ) was measured from the soil profile. On 6 Mar. 2013, SP and EF had  $\text{CO}_2$  concentrations that were 2.4 times smaller ( $p = 0.007$ ) than both HLF and LF treatments ( $13.2 \text{ mL L}^{-1}$ ), when considered across all soil depths. Across all sample dates, the highest ( $p < 0.001$ )  $\text{CO}_2$  concentrations were measured at 50 cm ( $7.1 \text{ mL L}^{-1}$ ), which was more than twice as great as concentrations at 15 cm and 43% more than those at 30 cm. The significant Treatment x Depth interaction ( $p < 0.001$ ) was as a result of the LF treatment having the highest ( $p = 0.01$ ) concentrations on 6 Mar. 2013 at 15 cm, yet the highest concentrations at 30 cm were measured from HLF treatment on this day. Concentrations at 15 cm were at their highest ( $p < 0.001$ ) on 6 Mar. 2013 ( $12.0 \text{ mL L}^{-1}$ ) after increasing by 100% in only one week. The next highest  $\text{CO}_2$  concentrations were on 13 Mar. 2013 ( $9.1 \text{ mL L}^{-1}$ ), which quickly became the lowest concentrations ( $1.1 \text{ mL L}^{-1}$ ) only five days later on 18 Mar. 2013.  $\text{CO}_2$  concentrations on 13 Mar. 2013 were twice as great as any other date at 30 cm. The lowest  $\text{CO}_2$  concentrations at 50 cm were measured during the month of Feb. and the highest during the first half of Apr.





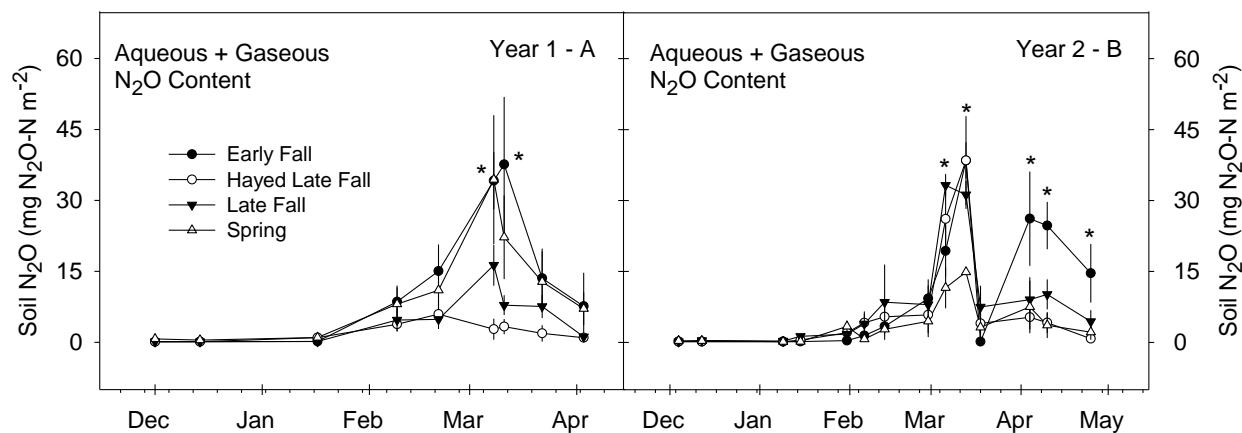
**Fig. 4.3.** Soil profile CO<sub>2</sub> concentrations at 15 (A, B), 30 (C, D), 50 (E, F) cm from Dec. to Apr. in Year 1 and Dec. to May in Year 2. Take note of the different scale on the Y-axis in panel E. Following a significant Treatment x Time interaction ( $p \leq 0.05$ ) differences among treatments among sample dates are indicated with an asterisk. Error bars represent the SEM ( $n=3$ ).

#### 4.5.4 Soil profile N<sub>2</sub>O content

There was no overall difference in N<sub>2</sub>O content (mg N<sub>2</sub>O-N m<sup>-2</sup>) when all depths are summed together among treatments when considered across all sample dates ( $p=0.09$ ) in Year 1 (Fig. 4.4 A). Across all treatments, the highest ( $p<0.001$ ) N<sub>2</sub>O content was measured on Mar. 6 2012, as compared to the lowest contents measured in Dec., Jan. and Apr. The Treatment x Time

interaction was significant ( $p=0.001$ ) when the highest  $\text{N}_2\text{O}$  content on 6 Mar. 2012 was measured in both the EF and SP treatments. On 11 Mar. 2012, the EF treatment had the highest  $\text{N}_2\text{O}$  content ( $37.62 \text{ mg N}_2\text{O-N m}^{-2}$ ) that was 41, 91, 80% greater than SP, Hayed, and LF treatments, respectively.

Across all sample dates in Year 2, there was no overall difference ( $p=0.21$ ) in  $\text{N}_2\text{O}$  content ( $\text{mg N}_2\text{O-N m}^{-2}$ ) among treatments (Fig. 4.4 B). Differences in  $\text{N}_2\text{O}$  content among treatments became evident on 6 Mar. 2013, when Hayed and LF treatments were 92% greater than both the EF and SP treatments that had a mean  $\text{N}_2\text{O}$  content of  $15.44 \text{ mg N}_2\text{O-N m}^{-2}$ . The highest  $\text{N}_2\text{O}$  content across all treatments was measured on 13 Mar. 2013, with a mean of  $30.8 \text{ mg N}_2\text{O-N m}^{-2}$ . The SP treatment had the lowest  $\text{N}_2\text{O}$  content on 13 Mar. 2013 when compared to all other treatments that had at least a two-fold increase over SP. Five days later, total  $\text{N}_2\text{O}$  content decreased to  $3.7 \text{ mg N}_2\text{O-N m}^{-2}$  and was among the lowest of all sample dates. Through the month of Apr., the highest  $\text{N}_2\text{O}$  contents were measured from the EF treatment as compared to all others.



**Fig. 4.4.** The sum of gaseous and aqueous  $\text{N}_2\text{O}$  content ( $\text{mg N}_2\text{O-N m}^{-2}$ ) within the soil profile (7.5–57.5 cm) in Year 1 (A) and Year 2 (B). Following a significant Treatment x Time interaction ( $p \leq 0.05$ ), differences among treatments across sample dates are indicated with an asterisk. Error bars represent the SEM ( $n=3$ ).

#### 4.5.5 Change in soil $\text{N}_2\text{O}$ content with surface flux

At the beginning of Feb. in Year 1,  $\text{N}_2\text{O}$  content within the soil profile started to increase (Table 4.1), with the largest increase happening from 21 Feb. to 8 Mar. 2012 in all treatments ( $18.0 \text{ mg N}_2\text{O-N m}^{-2}$ ). The only exception occurred in the HLF treatment, which decreased by  $3.2 \text{ mg N}_2\text{O-N m}^{-2}$  during this period. On 11 Mar. 2012, the SP and LF treatments had decreases

of 12.2 and 8.5 mg N<sub>2</sub>O-N m<sup>-2</sup>, respectively. This was in contrast to the EF treatment that did not see a decrease in N<sub>2</sub>O content until 22 Mar. 2012. With the exception of HLF and SP treatments, N<sub>2</sub>O contents (mg N<sub>2</sub>O m<sup>-2</sup>) were comparable to N<sub>2</sub>O emissions measured from the soil surface. Surface N<sub>2</sub>O emissions measured from Jan. to Apr. from the HLF treatment were an order of magnitude larger than the amount of N<sub>2</sub>O measured within the soil profile. Furthermore, SP tillage had N<sub>2</sub>O emissions that were nearly twice as large as the amount of N<sub>2</sub>O that had accumulated within the soil profile.

**Table 4.2.** The change in soil profile (7.5 to 57.5 cm) N<sub>2</sub>O mass (mg N<sub>2</sub>O-N m<sup>-2</sup>) between sample dates in Year 1.

| Date   | Treatments                               |                 |               |                |
|--|--|-----------------|---------------|----------------|
|  | Early Fall                               | Hayed Late Fall | Late Fall     | Spring         |
| ----- mg N <sub>2</sub> O-N m <sup>-2</sup> -----              |  |                 |               |                |
| 01-Dec-11  | 0.13 <sup>†</sup>                        | 0.11            | 0.12          | 0.71           |
| 14-Dec-11  | 0.01                                     | 0.01            | 0.01          | -0.23          |
| 17-Jan-12  | 0.03                                     | 0.86            | 0.05          | 0.54           |
| 09-Feb-12  | 8.41                                     | 2.82            | 4.52          | 7.05           |
| 21-Feb-12  | 6.48                                     | 2.14            | 0.16          | 2.93           |
| 08-Mar-12  | 19.11                                    | -3.17           | 11.45         | 23.29          |
| 11-Mar-12  | 3.45                                     | 0.54            | -8.45         | -12.2          |
| 22-Mar-12  | -24.09                                   | -1.43           | -0.27         | -9.4           |
| 03-Apr-12  | -5.9                                     | -0.93           | -6.41         | -5.7           |
| ----- Jan.-Apr. (mg N <sub>2</sub> O-N m <sup>-2</sup> ) ----- |  |                 |               |                |
| Surface N <sub>2</sub> O emissions                             | 19.25 (4.9) <sup>‡</sup> ab <sup>§</sup> | 15.36 (7.9) ab  | 11.89 (6.0) b | 47.48 (14.7) a |
| <i>p</i> -value  | 0.04                                     |                 |               |                |

<sup>†</sup> Positive values represent an accumulation, and negative values represent a decrease in N<sub>2</sub>O within the soil depth (7.5–57.5 cm) relative to the previous sample date.

<sup>‡</sup> Surface N<sub>2</sub>O emissions discussed in Chapter 3 are included here for comparison. Values in brackets represent the SEM.

<sup>§</sup> Means within rows with the same lower case letters are not significantly different at the  $p \leq 0.05$  level of probability.

In Year 2, there was a rapid increase in N<sub>2</sub>O at the beginning of Mar. in all treatments with increases over the next two weeks of 10.4, 23.2, 29.2, and 32.7 in the SP, LF, EF, and HLF treatments, respectively (Table 4.2). Between Mar. 13 and 18, 2013, there was a rapid decrease in N<sub>2</sub>O content in all treatments with the biggest change happening in EF (38.29 mg N<sub>2</sub>O-N m<sup>-2</sup>), and the smallest in the SP treatment with a decrease of 11.71 mg N<sub>2</sub>O-N m<sup>-2</sup>. In contrast to all other treatments, the EF treatment accumulated 26 mg N<sub>2</sub>O-N m<sup>-2</sup> from 18 Mar. to 4 Apr. 2013, as compared to only 2.4±1.7 mg N<sub>2</sub>O-N m<sup>-2</sup> in all other treatments. The decrease in soil N<sub>2</sub>O in

the EF treatment was similar to N<sub>2</sub>O emissions measured at the soil surface. Emissions of N<sub>2</sub>O in the SP treatment were less than what had accumulated in the soil profile, while surface emissions from Jan. to Apr. were twice as large as those that were measured in the soil profile for both the HLF and LF treatments.

**Table 4.3.** The change in soil profile (7.5 to 57.5 cm) N<sub>2</sub>O mass (mg N<sub>2</sub>O-N m<sup>-2</sup>) between sample dates in Year 2.

| Date                               | Treatments   |                 |                |             |
|------------------------------------|--|-----------------|----------------|-------------|
|                                    | Early Fall   | Hayed Late Fall | Late Fall      | Spring      |
|                                    | ----- mg N <sub>2</sub> O-N m <sup>-2</sup> -----              |                 |                |             |
| 04-Dec-12                          | 0.19 <sup>†</sup>  | 0.13            | 0.14           | 0.32        |
| 12-Dec-12                          | -0.02  | 0.01            | 0.25           | 0.01        |
| 09-Jan-13                          | 0.01   | 0.01            | -0.1           | -0.17       |
| 15-Jan-13                          | 0.01   | 0.32            | 0.98           | 0.02        |
| 31-Jan-13                          | 0.19   | 1.57            | 0.49           | 3.2         |
| 06-Feb-13                          | 1.07   | 2.11            | 2.17           | -2.68       |
| 13-Feb-13                          | 1.94   | 1.26            | 4.58           | 2.07        |
| 28-Feb-13                          | 5.9  | 0.39            | -0.52          | 1.7         |
| 06-Mar-13                          | 10.05  | 20.29           | 25.26          | 7.08        |
| 13-Mar-13                          | 19.11  | 12.38           | -2.02          | 3.33        |
| 18-Mar-13                          | -38.29   | -34.45          | -23.73         | -11.71      |
| 04-Apr-13                          | 26.00  | 1.3             | 1.56           | 4.34        |
| 10-Apr-13                          | -1.46  | -1.14           | -1.22          | -3.88       |
| 25-Apr-13                          | -10.08   | -3.4            | -0.82          | -1.46       |
| Surface N <sub>2</sub> O emissions | ----- Jan.-Apr. (mg N <sub>2</sub> O-N m <sup>-2</sup> ) ----- |                 |                |             |
|                                    | 35.29 (11.7) <sup>‡</sup> a <sup>§</sup>                       | 62.57 (30.0) a  | 52.76 (21.6) a | 3.6 (1.2) b |
| <i>p</i> -value                    | < 0.001  |                 |                |             |

<sup>†</sup> Positive values represent an accumulation, and negative values represent a decrease in N<sub>2</sub>O within the soil depth (7.5–57.5 cm) relative to the previous sample date.

<sup>‡</sup> Surface N<sub>2</sub>O emissions discussed in Chapter 3 are included here for comparison. Values in brackets represent the SEM.

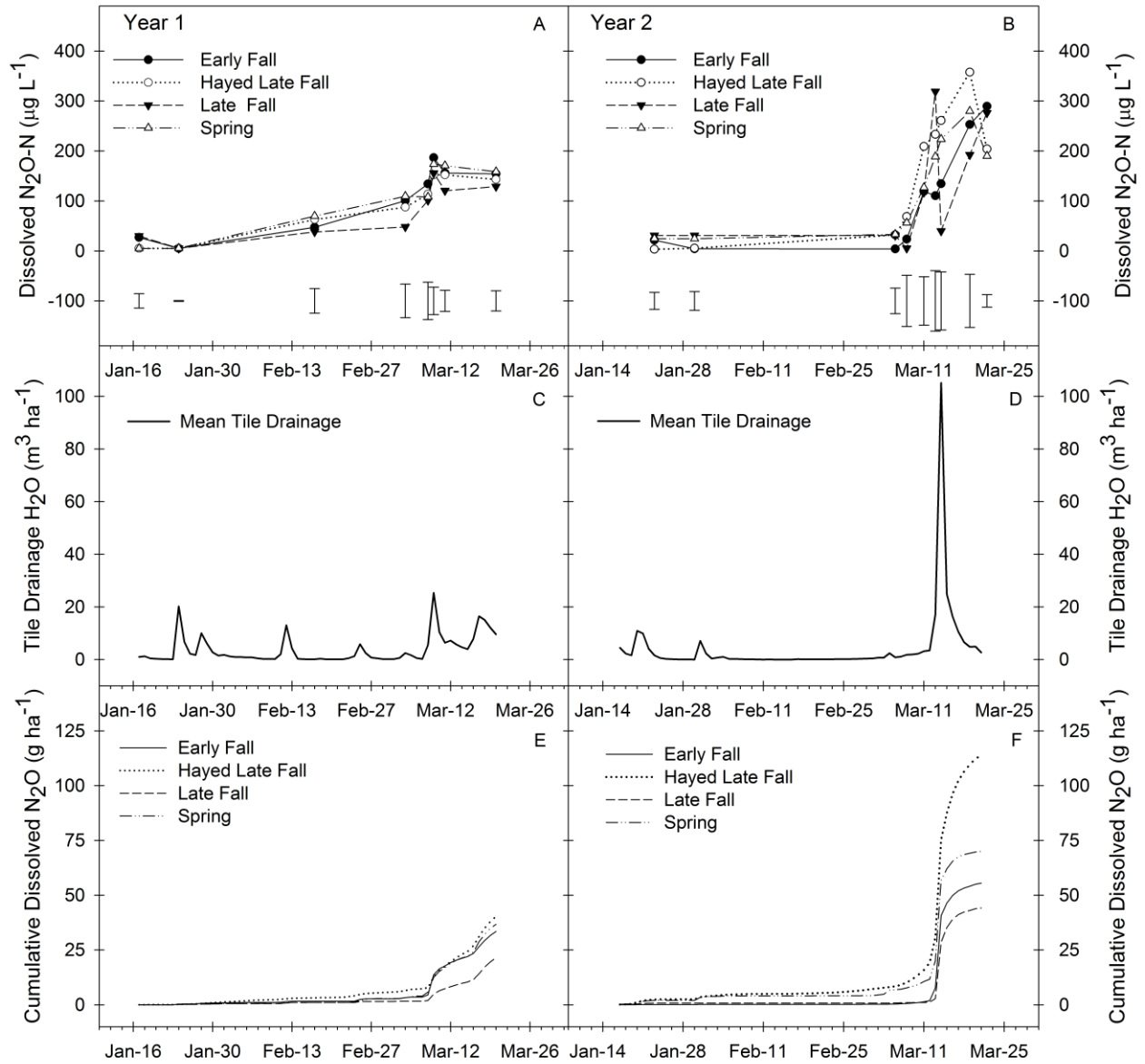
<sup>§</sup> Means within rows with the same lower case letters are not significantly different at the  $p \leq 0.05$  level of probability.

#### 4.5.6 Dissolved N<sub>2</sub>O in drainage water

Across all treatments, dissolved N<sub>2</sub>O concentrations in tile drainage water from Year 1 (Fig. 4.5 A) were different over all sampling dates ( $p < 0.001$ ), but there was no overall effect of treatment ( $p = 0.7$ ), nor was the interaction significant ( $p = 0.99$ ). The highest concentration was measured on 9 Mar. 2012 with a mean ( $\pm$ SEM) of  $167 \pm 16$   $\mu$ g N<sub>2</sub>O-N L<sup>-1</sup> across treatments, as compared to the lowest concentrations measured in Jan. and Feb. with a range of  $5 \pm 0.5$  to  $55 \pm 14$   $\mu$ g N<sub>2</sub>O-N L<sup>-1</sup>. Cumulative dissolved N<sub>2</sub>O-N collected from drainage tiles in Year 1 (Fig. 4.5 E)

was not different among treatments ( $p=0.64$ ) with a range of 22 to 41 g N<sub>2</sub>O-N ha<sup>-1</sup> from 17 Jan. 2012 to 20 Mar. 2012 (64 d). Mean drainage volume across all plots during this time period was 230±139 m<sup>3</sup> H<sub>2</sub>O ha<sup>-1</sup> (Fig. 4.5 C).

In Year 2, dissolved N<sub>2</sub>O concentrations (Fig. 4.5 B) were again not different among treatments ( $p= 0.72$ ), yet were different with time ( $p<0.001$ ), but without any interaction with treatment ( $p=0.33$ ). The highest dissolved N<sub>2</sub>O concentration was measured on 13 Mar. 2013 (269±30 µg N<sub>2</sub>O-N L<sup>-1</sup>) and was not different than all other concentrations measured in Mar. 2013 that ranged from 202±25 to 240±34 µg N<sub>2</sub>O-N L<sup>-1</sup>. The lowest concentrations were measured during the month of Jan. 2013 with a mean of 32±10mg N<sub>2</sub>O-N L<sup>-1</sup>. Cumulative N<sub>2</sub>O losses in drainage water (Fig. 4.5 F) were again not different among treatments ( $p= 0.18$ ) with a range of 43 to 115 g N<sub>2</sub>O-N ha<sup>-1</sup> from 19 Jan. 2013 to 23 Mar. 2013 (64 d). Overall, drainage volume across all plots was 266±84 m<sup>3</sup> H<sub>2</sub>O ha<sup>-1</sup> (Fig. 4.5 D).



**Fig. 4.5.** Dissolved  $N_2O-N$  ( $\mu g L^{-1}$ ) in drainage water (A, B), mean drainage water volume ( $m^3 ha^{-1}$ ) from all plots (C, D), and cumulative dissolved  $N_2O-N$  ( $g ha^{-1}$ ) (E, F). Error bars in (A, B) represent the average SEM for all treatments ( $n=3$ ).

#### 4.5.7 Pearson correlation coefficients of soil parameters

Linear relationships of CO<sub>2</sub> and N<sub>2</sub>O soil concentrations at multiple depths, daily N<sub>2</sub>O and CO<sub>2</sub> emissions, and dissolved N<sub>2</sub>O concentrations in drainage water on six dates between Jan. and Apr. were selected in each year and were evaluated using Pearson's correlation coefficients (Table 4.3). As observed in Chapter 3, emissions of CO<sub>2</sub> and N<sub>2</sub>O at the soil surface were highly correlated ( $r=0.81$ ;  $p<0.001$ ;  $n=46$ ). Soil concentrations of CO<sub>2</sub> and N<sub>2</sub>O in space and over time were positively related at 30- ( $r=0.76$ ;  $p<0.001$ ;  $n=46$ ) and 15-cm ( $r=0.70$ ;  $p<0.001$ ;  $n=46$ ) as well. Temporal variations in N<sub>2</sub>O concentrations at 30 cm were closely related to both 15- ( $r=0.72$ ;  $p<0.001$ ;  $n=46$ ) and 50-cm ( $r=0.65$ ;  $p<0.001$ ;  $n=46$ ), while CO<sub>2</sub> at 30 cm was most related to CO<sub>2</sub> at 15 ( $r=0.49$ ;  $p<0.001$ ;  $n=46$ ) when compared to 50 cm ( $r=0.30$ ;  $p<0.04$ ;  $n=46$ ).

The size of the daily N<sub>2</sub>O emission event had strong positive relationships with N<sub>2</sub>O concentrations at 30- ( $r=0.52$ ;  $p<0.001$ ;  $n=46$ ) and 50-cm ( $r=0.45$ ;  $p=0.001$ ;  $n=46$ ), but no relationship was found for the N<sub>2</sub>O concentration at 15 cm ( $r=0.01$ ;  $p=0.99$ ;  $n=46$ ). Interestingly, there were no significant relationships of daily CO<sub>2</sub> emissions with 15 cm CO<sub>2</sub> or N<sub>2</sub>O concentrations either, yet Soil CO<sub>2</sub> concentrations at 30 cm were positively related to surface N<sub>2</sub>O flux ( $r=0.42$ ;  $p=0.003$ ;  $n=46$ ). Another relationship indicating the temporal relationships of winter N loss is the increase in surface flux events with the concentration of N<sub>2</sub>O dissolved in drainage water ( $r=0.44$ ;  $p=0.002$ ;  $n=46$ ). Furthermore, soil N<sub>2</sub>O concentrations were positively related to dissolved N<sub>2</sub>O concentrations collected in drainage water for N<sub>2</sub>O at soil depths of 50- ( $r=0.58$ ;  $p<0.001$ ;  $n=46$ ) and 30-cm ( $r=0.43$ ;  $p=0.002$ ;  $n=46$ ).

**Table 4.4.** Pearson correlation coefficients (*r*) among soil gas concentrations and N<sub>2</sub>O losses to air and drainage water on 12 sample days from Jan. 1 to Apr. 1 in Years 1 and 2.

|  | Daily N <sub>2</sub> O<br>surface<br>flux <sup>†</sup> | Daily CO <sub>2</sub><br>surface<br>flux | ----- Soil gas -----        |                             |                             |                             |                             |                             |
|--|--|--|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|  |  |  | [CO <sub>2</sub> ]<br>15 cm | [N <sub>2</sub> O]<br>15 cm | [CO <sub>2</sub> ]<br>30 cm | [N <sub>2</sub> O]<br>30 cm | [CO <sub>2</sub> ]<br>50 cm | [N <sub>2</sub> O]<br>50 cm |
| Dissolved N <sub>2</sub> O<br>(µg N <sub>2</sub> O-N L <sup>-1</sup> ) | 0.44***  | 0.34*                                    | 0.04                        | 0.25                        | 0.22                        | 0.43**                      | -0.06                       | 0.58***                     |
| Daily N <sub>2</sub> O<br>surface flux                                 |  | 0.81***                                  | -0.002                      | 0.000                       | 0.42**                      | 0.52***                     | 0.05                        | 0.45***                     |
| Daily CO <sub>2</sub><br>surface flux                                  |  |  | -0.15                       | -0.15                       | 0.24                        | 0.27                        | 0.04                        | 0.23                        |
| Soil [CO <sub>2</sub> ]<br>15 cm                                       |  |  |                             | 0.70***                     | 0.49***                     | 0.52***                     | 0.37**                      | 0.27                        |
| Soil [N <sub>2</sub> O]<br>15 cm                                       |  |  |                             |                             | 0.47***                     | 0.72***                     | -0.04                       | 0.34*                       |
| Soil [CO <sub>2</sub> ]<br>30 cm                                       |  |  |                             |                             |                             | 0.76***                     | 0.30*                       | 0.27                        |
| Soil [N <sub>2</sub> O]<br>30 cm                                       |  |  |                             |                             |                             |                             | 0.08                        | 0.65***                     |
| Soil [CO <sub>2</sub> ]<br>50 cm                                       |  |  |                             |                             |                             |                             |                             | 0.16                        |

<sup>†</sup> Dissolved N<sub>2</sub>O (µg N<sub>2</sub>O-N L<sup>-1</sup>) refers to drainage water N<sub>2</sub>O concentration on the day of sampling. Daily N<sub>2</sub>O and CO<sub>2</sub> flux refers to the interpolated values for that day. Soil gas refers to N<sub>2</sub>O and CO<sub>2</sub> concentrations within the soil profile.

<sup>‡</sup> Six sampling events in each year (Jan.–Apr.) and 4 treatments created a total sample size of 48 (*n* = 46) for the correlation matrix. \* Significant at the 0.05, \*\* 0.01, and \*\*\* 0.001 levels of probability.



## 4.6 DISCUSSION

### 4.6.1 *Soil profile N<sub>2</sub>O and CO<sub>2</sub> concentrations and content*

Elevated N<sub>2</sub>O concentrations within the soil profile during frozen soil conditions are caused by increased production without further reduction to N<sub>2</sub> and the lack of diffusion or mass-flow out of the soil profile. Before an increase in N<sub>2</sub>O within the soil profile in this study, there were several rapid thaw and re-freeze events in Jan. and Feb in both years, although these were greater in magnitude in Year 2 (Fig. 4.1). Similar relationships between soil temperature and increasing soil N<sub>2</sub>O concentrations in soils that experience freezing and thawing conditions have been observed frequently (Maljanen et al., 2007; Yanai et al., 2011; Risk et al., 2014). Snowmelt or precipitation that freezes at or near the soil surface fill soil pores with ice that effectively seals the soil profile from the atmosphere and has been suggested to be a mechanism that initiates soil N<sub>2</sub>O accumulation (Burton and Beauchamp, 1994; Risk et al., 2014). Soil concentrations need to be considered with respect to surface conditions, as frozen soil layers close to the surface might limit the upward diffusion and downward movement of soil water. The highest N<sub>2</sub>O concentrations measured within the soil profile in this study (ca., 150  $\mu\text{L L}^{-1}$ ) were similar to those measured by Risk et al. (2014) near the soil surface, but our measurements were an order of magnitude larger at 30 and 50 cm as compared to those measured by Risk et al. (2014). However, this trend was not observed in all blocks in our study, as indicated by the large variation during many sampling dates. Burton and Beauchamp (1994) also described a high amount of variability in soil N<sub>2</sub>O concentrations; although field variability is anticipated, the magnitude of differences among treatments in our study were somewhat surprising. However, the ratio of NO<sub>3</sub><sup>-</sup> to available C together with the aeration status in soil is well known to affect which product of denitrification will be dominant. The timing and season of GMr incorporation would therefore affect the availability of substrate needed during denitrification, which would allow the production of N<sub>2</sub>O to vary among treatments. The varied effects of soil freezing

between years and treatments could also help explain the high variance in soil gas concentrations.

Soil N<sub>2</sub>O contents before spring thaw differed among treatments in both Year 1 and 2, but the trend among treatments was not consistent. In particular, the undisturbed GMr treatment (SP) had the highest N<sub>2</sub>O content in Year 1 and the lowest in Year 2; a trend that was observed for surface emissions as well (Chapter 3). Indeed, the positive relationship of N<sub>2</sub>O concentrations at 30 and 50 cm with daily surface flux (Table 4.4) indicates that N<sub>2</sub>O dynamics within the soil profile related to similar differences in daily N<sub>2</sub>O flux at the soil surface. The very weak relationships between surface flux and both N<sub>2</sub>O and CO<sub>2</sub> concentrations at the 15 cm soil depth was surprising, but may reflect the fact that N<sub>2</sub>O at 15 cm was already emitted at the soil surface, had experienced a reduction to N<sub>2</sub>, or were transported to lower soil depths with moving soil water. When the amount of N<sub>2</sub>O within the soil profile is scaled up from m<sup>-2</sup> to ha<sup>-1</sup> for a comparison with surface emissions that occurred during the month of Mar. (spring thaw), the relationship between soil accumulation and emission at the soil surface is again inconsistent among treatments in each year. This was true for all treatments except EF that had increased soil profile N<sub>2</sub>O content and then reductions that were consistently greater than the measured emissions at the soil surface by 90 and 182 g N<sub>2</sub>O-N ha<sup>-1</sup> in Year 1 and 2, respectively. This pattern was also seen in the SP treatment in Year 2. However, the treatments with the largest spring thaw N<sub>2</sub>O emissions in Year 1 and 2 had more N<sub>2</sub>O emitted from the soil surface than what had accumulated in the soil profile by a factor of 50% for SP in Year 1 as compared to 36 and 41% for HLF and LF treatments in Year 2. The change in profile N<sub>2</sub>O content and the amount emitted from the soil surface during spring thaw was similar for HLF and LF in Year 1 with surface emissions of 41 and 102 g N<sub>2</sub>O-N ha<sup>-1</sup>, respectively. Hydrological controls and substrate availability were different among years and could help explain why there were inconsistencies among treatment effects on soil N<sub>2</sub>O content and emissions from the soil surface when compared over two years. Overall, N<sub>2</sub>O concentrations within the soil profile were a good indicator of the potential for N<sub>2</sub>O emissions at the soil surface; treatments with the greatest surface emissions at spring thaw also had the highest profile N<sub>2</sub>O concentrations.

Because concentrations of CO<sub>2</sub> and N<sub>2</sub>O in the soil atmosphere are a result of microbially-mediated processes (Amundson and Davidson, 1990), it should not be too much of a surprise that CO<sub>2</sub> and N<sub>2</sub>O concentrations were highly correlated at 15 ( $r = 0.70$ ;  $p < 0.001$ ;  $n = 46$ )

and 30 cm ( $r=0.76$ ;  $p<0.001$ ;  $n=46$ ) soil depths. Because of the very high concentrations of both of these gases before spring thaw, there is good evidence of limited diffusion from the soil profile to the atmosphere. The similar dynamics of CO<sub>2</sub> and N<sub>2</sub>O concentrations within the soil profile as well as emissions from the soil surface over time suggests that soil physical properties have a strong control on the overall potential for surface emissions and the duration of increasing and decreasing soil gases. Meanwhile, the difference among treatments in each year suggests that more of the biological controls and substrate availability will dictate the magnitude of these changes. Cropping systems with high C inputs have been shown to have high N<sub>2</sub>O emissions as a result of the localized anaerobic conditions created by the increase in biological activity (Petersen et al., 2013), however it is unclear what the effect would be in cold soils with limited soil gas exchange with air above the soil surface. Furthermore, the expression and activity of N<sub>2</sub>O reductase enzyme in cold soils has been identified as being a key process that may lead to N<sub>2</sub>O being the dominant product of denitrification (Smith et al., 2010; Wertz et al., 2013; Németh et al., 2014; Tatti et al., 2014).

Changes in surface conditions due to timing of GMr tillage may have other effects on the biological community. Plant cover has the ability to trap more snow as compared to bare soil surfaces, which lessens the effects of air temperature fluctuations and reduces the extent of soil frost. The actions of soil freezing and thawing can break up soil aggregates that increase substrate availability that was otherwise physically protected within the aggregate. Available C and N also increase as a result of plant and soil animal cell lysis, which is known to occur following a freeze thaw-cycle (Cleavitt et al., 2008; Elliott and Henry, 2009). Furthermore, restricted outward diffusion of soil gas, as indicated by high gas concentrations, also implies that the inward diffusion of O<sub>2</sub> is restricted, which would create anaerobic conditions that are necessary for denitrification and N<sub>2</sub>O production (Amundson and Davidson, 1990). While the pattern of soil freezing events was similar across treatments, soil temperatures were consistently lower in both the HLF and LF treatments and intermediate for the EF, when compared to SP, at all soil depths in Year 2. This result was surprising, but was likely a factor of the Nov. plowing that occurred in both of these treatments and the influence that plant cover has on the ability to trap snow and insulate the soil from freezing conditions. Air-filled porosity and the size of soil pores also influence the extent and degree of soil freezing (Parkin et al., 2013). Surface conditions and a change in the distribution and size of soil pores would be modified by inversion

tillage and could explain why there was a difference in soil freezing among treatments at all soil depths in Year 2 (Fig. 4.1).

#### 4.6.2 *Dissolved N<sub>2</sub>O in tile drainage flow*

Because GMr tillage treatments only represented 25% of the area that contributed to water collected from drainage tiles, it is not surprising that there were no differences in Treatments in either year of study (Fig. 4.5 E, F). The variability in dissolved N<sub>2</sub>O concentrations among treatments (Fig. 4.5 A, B) that was evident in both years is not necessarily due solely to differences in the timing of clover incorporation, but more of an indication of the variable site conditions that influence both N<sub>2</sub>O production and the hydrological properties. However, the similar timing for increasing dissolved N<sub>2</sub>O concentrations at spring thaw with the decrease in soil N<sub>2</sub>O concentrations from GMr plots in both study years indicates that this rarely measured pathway for N<sub>2</sub>O losses can be significant. The greatest dissolved N<sub>2</sub>O concentrations were measured on the same day as the largest drainage events in both Year 1 and 2. In Year 1, 59% of the total drainage volume from Jan. to Apr. occurred during the month of Mar. as compared to 79% in Year 2. Nitrous oxide contents in drainage water from Year 2 were nearly double those in Year 1 because of greater drainage, and higher overall concentrations during spring thaw.

In Year 2 of the study, we started measuring dissolved N<sub>2</sub>O losses earlier in the fall with mean N<sub>2</sub>O losses being 44 g N<sub>2</sub>O-N ha<sup>-1</sup> from 16 Oct. to 1 Jan (data not shown). Although there was 5-fold increase in dissolved N<sub>2</sub>O in Year 2 when compared to Year 1 when a 184 d sampling period was considered, much of this difference is due to the longer sampling period and an increase in drainage water volume at spring thaw. When the amount of dissolved N<sub>2</sub>O in drainage water was calculated on a daily or water volume basis there were no statistical differences among years (data not shown). Dissolved N<sub>2</sub>O concentrations were highest at spring thaw, but low concentrations with high amounts of drainage volume during the late fall and early winter will contribute to annual N<sub>2</sub>O losses in drainage water. Another point to consider is the fact that N<sub>2</sub>O concentrations in drainage water had not declined to their lowest levels at the end of our study periods, and therefore, there is a possibility for more N<sub>2</sub>O losses that were unaccounted for in the spring and early summer during this study.

The positive relationship (Table 4.4) of dissolved N<sub>2</sub>O concentrations with N<sub>2</sub>O concentrations at 30- and 50 cm ( $r=0.43-58$ ;  $p<0.01$ ;  $n=46$ ) and surface N<sub>2</sub>O flux ( $r=0.44$ ;  $p=0.002$ ;  $n=46$ ) indicate that at this site the depth and magnitude of N<sub>2</sub>O concentrations through time are an effective indicator of the timing and potential for N<sub>2</sub>O losses from the soil surface and N<sub>2</sub>O dissolved in drainage water. During periods of high drainage and elevated N<sub>2</sub>O concentrations within the soil profile, as observed during Mar. in each year at this study site, there was also an increased risk for dissolved N<sub>2</sub>O in drainage water. It is unclear if effective and rapid water drainage from the installation of tile drainage has the potential to reduce surface N<sub>2</sub>O flux from the soil surface, or merely transfers the emissions to the drainage ditch or outlet. Further investigation of the relationship of improved drainage and related spring thaw N<sub>2</sub>O losses is needed.

Most of the studies assessing dissolved N<sub>2</sub>O losses from agricultural areas have been focused during the growing season, or do not have extended freezing and thawing periods in which high concentrations of N<sub>2</sub>O can accumulate under a restricted layer. Heincke and Kaupenjohann (1999) reviewed N<sub>2</sub>O-N concentrations in drainage water that had a range from 25 to 132  $\mu\text{g N}_2\text{O-N L}^{-1}$ . Reay et al. (2004a) reported comparatively smaller N<sub>2</sub>O-N concentrations in drainage water (2–4  $\mu\text{g N}_2\text{O-N L}^{-1}$ ) that resulted in an annual loss of 107 g N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>, and indirect N<sub>2</sub>O emissions via drainage water were considered to be insignificant in Scottish agricultural systems. From Jan. to Apr. in our study, there was a mean of 33±13 and 70±34 g N<sub>2</sub>O-N ha<sup>-1</sup> lost as dissolved N<sub>2</sub>O across all treatments in Year 1 and 2, respectively, which was 14 and 18% of N<sub>2</sub>O-N emitted from the soil surface during the same period (Tables 4.1 and 4.2). Reay et al. (2004b) measured dissolved N<sub>2</sub>O losses to be <1% of the N<sub>2</sub>O emitted at the soil surface over 30 days following spring fertilization of a barley field in the U.K, but likely did not have the same increase in soil N<sub>2</sub>O concentrations that were measured here prior to spring thaw. A lysimeter study from an intensively cropped volcanic ash soil in Japan reported annual N<sub>2</sub>O losses in drainage water of 114 g N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>, which was in comparison nearly half of the measured total emissions from the soil surface (Minamikawa et al., 2013). Therefore, N<sub>2</sub>O losses from drainage water can be considerable in some systems, especially when considered over the entire year. Additionally, diffusion restrictions imposed by frozen soil layers, as seen in this study, increases the likelihood of rapid N<sub>2</sub>O losses in drainage water. Finally, cumulative dissolved N<sub>2</sub>O and maximum daily flux losses (g N<sub>2</sub>O-N ha<sup>-1</sup>) among

treatments were similar to each other, and were occurring concurrently at the peak of the spring thaw drainage event.

#### **4.7 Conclusions**

Nitrous oxide dissolved in drainage water represents a  $\text{N}_2\text{O}$  loss pathway that could lead to increased  $\text{N}_2\text{O}$  emissions to the atmosphere, especially when fields have drainage tiles installed. There was a mean of 33 and 70 g  $\text{N}_2\text{O}\text{-N ha}^{-1}$  measured in drainage water in Year 1 and 2, respectively, which represents a loss of  $\text{N}_2\text{O}$  from these fields that were comparable to the highest daily emission events that occurred at spring thaw. The buildup of  $\text{N}_2\text{O}$  within the soil profile was similar in magnitude between years with a maximum of around 40 mg  $\text{N}_2\text{O}\text{-N m}^{-2}$  in both years. The disappearance of  $\text{N}_2\text{O}$  within the soil profile was rapid in both years and occurred at the peak of the spring thaw drainage, but then climbed again when drainage slowed. Significant correlations indicated that the disappearance of  $\text{N}_2\text{O}$  from within the soil profile was also closely timed with  $\text{N}_2\text{O}$  emissions at the soil surface and the increase in dissolved  $\text{N}_2\text{O}$  concentrations in drainage water, yet GMr incorporation treatments had varying effects on  $\text{N}_2\text{O}$  production within the soil profile and subsequent losses to the environment from year to year. However, the change in  $\text{N}_2\text{O}$  content from within the soil profile occurred over a shorter period of time in Year 2 as compared to Year 1 due to the increase in drainage in Year 2, and the accumulation of  $\text{N}_2\text{O}$  within the soil profile that occurred later in winter. The highest  $\text{N}_2\text{O}$  contents among treatments also coincided with the highest emissions from the surface at spring thaw; an indication that the concentration of  $\text{N}_2\text{O}$  within the soil profile is also good predictor of  $\text{N}_2\text{O}$  production, and the potential for emissions from the soil surface or being emitted in drainage water during spring thaw.

## **5. SEASONAL VARIATION HAS A GREATER INFLUENCE ON SOIL N AVAILABILITY AND CROP N UPTAKE THAN THE TIMING OF GREEN MANURE TILLAGE IN ORGANIC WHEAT PRODUCTION**

### **5.1 Preface**

One of the greatest challenges for agricultural production systems that use green manures (GMr) for soil fertility is to manage the rate and amount of  $\text{NO}_3^-$  production to match requirements of the following cash crop. Soil N supply (SNS) becomes unsynchronized with plant uptake, due to atypical spring soil conditions or crop failure, which will result in elevated soil  $\text{NO}_3^-$  concentrations that then become susceptible to N losses, such as  $\text{NO}_3^-$  leaching and denitrification, especially in humid regions in Atlantic Canada. Emissions and dynamics of  $\text{N}_2\text{O}$  from the GMr phase of the rotation during the off-season were the foci of the previous research chapters, whereas in this chapter the focus is on the agronomic performance of the cash crop (wheat), SNS, associated in-season  $\text{N}_2\text{O}$ -N losses, plus annual  $\text{NO}_3^-$  leaching. I evaluated how fertility management systems (FMS) affected wheat N uptake and yield, with respect to in-season  $\text{N}_2\text{O}$  emissions. Two treatments are different in this chapter because of the addition of fertilizer in the EF treatment and manure application in HLF before wheat planting. Due to the nature of the experimental design, each phase of the 4-yr rotation was present in every year; thus, annual loss and monthly mean  $\text{NO}_3^-$  concentrations from subsurface drainage tiles are interpreted for the entire 4-yr crop rotation.

## 5.2 Abstract

A 2-yr study was conducted in Truro, NS, Canada to investigate potential fertility management systems (FMS) that provide the highest spring wheat yields with minimal  $\text{NO}_3^-$  leaching, and in-season  $\text{N}_2\text{O}$  emissions. Four FMS differed in the timing of green manure (GMr) tillage and the use of supplemental N before spring wheat planting as follows: (1): Sep. incorporated GMr and spring application of  $70 \text{ kg NH}_4\text{NO}_3 \text{ ha}^{-1}$  (Early Fall +  $\text{N}_{70}$ ; EF+ $\text{N}_{70}$ ); (2) Nov. incorporated GMr with GMr hay removed and spring application of manure at  $70 \text{ kg N ha}^{-1}$  (Hayed Late Fall +  $\text{M}_{70}$ ; HLF+ $\text{M}_{70}$ ); (3) Nov. incorporated GMr with no supplemental N (Late Fall; LF); (4) May incorporated GMr with no supplemental N (Spring; SP). There were no differences in wheat N uptake or grain yield among FMS in either year of study. However, there was a 76% increase ( $94.5 \pm 19.9$  to  $166 \pm 7.5 \text{ kg N ha}^{-1}$ ) in N uptake and wheat grain yield that was 2.5 times greater ( $+1583 \text{ kg ha}^{-1}$ ) in Year 2 than Year 1. Differences in N uptake between years were also supported by more soil available  $\text{N}_{\text{min}}$  in Year 2 and a 44% decrease in annual  $\text{NO}_3^-$  leaching ( $15.5 \text{ kg NO}_3^- \text{-N ha}^{-1}$ ), which was an indication of a soil N supply (SNS) that was better synchronized for plant uptake in Year 2. The 2-yr mean  $\text{NO}_3^-$ -N concentration in drainage water was greatest from the EF+ $\text{N}_{70}$  ( $10.7 \text{ mg NO}_3^- \text{-N L}^{-1}$ ) FMS during the fall and early spring, as compared to the other FMS that had a mean value of  $7.7 \text{ mg NO}_3^- \text{-N L}^{-1}$ . In-season  $\text{N}_2\text{O}$  emissions from the wheat crop did not differ among FMS, with the exception of an increase in  $\text{N}_2\text{O}$  on two sample dates following manure application in the HLF+ $\text{M}_{70}$  FMS in Year 1, but were otherwise low ( $0.09\text{--}0.87 \text{ kg N}_2\text{O-N ha}^{-1}$ ). Weather variation between years had a greater control on in-season SNS and overall N dynamics than FMS alone. There was no agronomic advantage of using supplemental N, especially when the additional costs for application and the potential for additional N losses are considered.

## 5.3 Introduction

The balance between sufficient N availability for plant growth while minimizing environmental N losses is a challenge in all agricultural systems, especially those that are managed organically. There have been numerous examples of reduced N losses in organic production systems that rely on organic N sources, as compared to conventional systems that rely



on mineral fertilizers for soil fertility (Drinkwater et al., 1998; Stopes et al., 2002; Eriksen et al., 2004). Furthermore, systems that rely on biologically fixed N as the main N input are characterized as having lower whole-farm GHG emissions per hectare (Drinkwater et al., 1998; Olesen et al., 2006), better soil C storage and sequestration (Hepperly et al., 2007; Zikeli et al., 2013), soil health (Lynch, 2014), and lower whole-farm energy use (Refsgaard et al., 1998; Lynch, 2009; Lynch et al., 2011). In contrast, yield-scaled N<sub>2</sub>O emissions are higher in organic systems when compared to conventional management (Skinner et al., 2014), and NO<sub>3</sub><sup>-</sup> leaching from red clover GMr can be higher than systems that rely only on mineral fertilizer application (Bergstrom and Kirchmann, 2004).

Total N inputs from GMr and animal manures used in organic management have been shown to be comparable to conventional systems (Berry et al., 2002), yet yields can be limited by N availability (Lynch et al., 2012). Nitrogen losses and the yield of organically managed cash crop rotations can be dramatically influenced by in-season soil N supply (SNS) (Koraseth et al., 2002), which is described as the sum of available N from the mineralization of soil organic matter, crop residues, and organic amendments in addition to any residual N from the previous growing season (Dessureault-Rompere et al., 2015). Because of year to year variation in soil temperature and water content, the timing of the SNS is difficult to predict (Griffin, 2008; Dessureault-Rompere et al., 2012). As a result, the timing of the SNS from GMr to the subsequent crop can become unsynchronized with crop uptake and results in lower yields, rather than being a result of low total N input (Berry et al., 2002). Management choices for GMr that could increase soil N uptake by the cash crop involve adjusting the timing and season of incorporation, growing for forage, or using the biomass for anaerobic digestion before applying it to the field (Lynch et al., 2012; Brozyna et al., 2013).

The transfer of N from GMr to a subsequent cash crop varies widely due to the legume species grown and the climate and soil type being studied. However, N inputs that are in the range of 150 to 300 kg N ha<sup>-1</sup> are possible (Berry et al., 2003). Potatoes in eastern Canada were supplied with 100 to 125 kg N ha<sup>-1</sup> following a legume (i.e., red clover or vetch) GMr (Lynch et al., 2012), which was comparable to a crop uptake of 99 kg N ha<sup>-1</sup> from potatoes supplied with N by an alfalfa/timothy plowdown in northeastern USA (Sharifi et al., 2008). Spring incorporation of GMr better protects against N losses outside of the growing season, but spring SNS for N demanding crops may not be well synchronized if soil mineralization and nitrification processes

are affected by soil conditions that either slow, or increase their rates prior to the planting of the cash crop. Furthermore, the timing of spring tillage can be problematic due to wet soil conditions that may limit field accessibility and delay the planting of the successive crop. In Atlantic Canada, SNS and the timing of N uptake by the successive crop may have to be managed by incorporating the GMr in the fall, or by including supplemental nitrogen sources in the spring before planting (i.e., fertilizer or manure) because of the shorter growing season.

Leaching of  $\text{NO}_3^-$  is controlled by N mineralization rates, crop uptake, manure or fertilizer additions, climate and soil types, and the timing of tillage (Dinnes et al., 2002). For example, given the combination of coarse textured soils and high fall precipitation that is characteristic of areas with Podzolic soils, the majority of the SNS comes directly from in-season mineralization (Zebarth et al., 2009). Agricultural systems in these types of soils and climate therefore have a greater risk of N leaching (De Jong et al., 2007; Zebarth et al., 2009), not only from high N application rates, but low crop uptake as well (Zebarth et al., 2009). However, there can be considerable  $\text{NO}_3^-$  production in the fall following legume residue incorporation (Sanderson and MacLeod, 1994; Dahlin et al., 2005), which also becomes susceptible to leaching. November soil profile (0–90 cm)  $\text{NO}_3^-$ -N content from a sandy loam Podzol in Atlantic Canada was greatest when red clover was incorporated with a moldboard plow in Sep. ( $82.7 \text{ kg N ha}^{-1}$ ), as compared to Oct. incorporation ( $41.0 \text{ kg N ha}^{-1}$ ), or was left undisturbed ( $20.3 \text{ kg N ha}^{-1}$ ) through the winter (Sanderson et al., 1999). The greatest  $\text{NO}_3^-$  leaching observed in a 6-yr crop rotation from Denmark, with varied animal stocking densities and manures, occurred when a legume/grass phase of the rotation was incorporated (Eriksen et al., 2004). Similarly, 20% less total N was leached in autumn when  $\text{NH}_4\text{NO}_3$  was spring applied rather than the spring incorporation of GMr in Sweden (Bergstrom and Kirchmann, 2004). The season and timing of GMr incorporation needs to be considered when the reduction of  $\text{NO}_3^-$  leaching is an objective of the agricultural system.

Similar to  $\text{NO}_3^-$  leaching, field management decisions that increase N use efficiency (NUE) and reduce residual soil mineral nitrogen (RSMN) in cropping systems are also critical for effective  $\text{N}_2\text{O}$  emission mitigation (Smith et al., 2008; Van Groenigen et al., 2010). Denitrification is the likely  $\text{N}_2\text{O}$  producing pathway following animal manure slurry application or legume GMr incorporation, but specific soil conditions and inputs of C and N will determine the proportion of  $\text{N}_2\text{O}$  produced during denitrification (i.e., molar ratio;  $\text{N}_2\text{O}:\text{N}_2+\text{N}_2\text{O}$ ). Drury et

al. (2008) reported the greatest field  $\text{N}_2\text{O}$  emissions were from monoculture corn ( $2.62 \text{ kg N ha}^{-1}$ ) as compared to  $1.03 \text{ kg N ha}^{-1}$  from a soybean–corn–wheat rotation. Large differences in N losses between the two systems were attributed to the high rates of fertilizer use, and the size of the RSMN pool in continuous corn. In contrast, Rochette et al. (2004) determined that soils fertilized with liquid manure had greater emissions over a short period of time, as compared to those systems that rely solely on mineral fertilizers for fertility. The triggers for these emissions were the high availability of  $\text{NH}_4^+$  and labile C in manure slurry coupled with precipitation events that increased water filled pore space (WFPS) in soil to levels that favored denitrification with a high  $\text{N}_2\text{O}:\text{N}_2+\text{N}_2\text{O}$  ratio (Chantigny et al., 1998).

Reducing environmental N loss is an important environmental goal for agricultural production systems; in addition, it can improve the economic performance of operations by reducing the cost of purchased N inputs in the form of inorganic fertilizers or manure (Roberts et al., 2008; Zebarth et al., 2009; Novak and Fiorelli, 2010). Legumes grown as GMr have the ability to supply sufficient N to successive crops, but there is a risk for N losses if the SNS becomes unsynchronized with crop demand (Koraseth et al., 2002). The objective of this study was to determine how four FMS using red clover as green manure for spring wheat production in Atlantic Canada affected spring wheat grain yield and N uptake along with in-season  $\text{N}_2\text{O}$  emissions and annual  $\text{NO}_3^-$  leaching from subsurface drainage tiles.

## 5.4 MATERIALS AND METHODS

### 5.4.1 Field site description and experimental design

All field data were collected at the Brookside experimental site for organic agriculture at Dalhousie University's Agricultural Campus, Truro, Nova Scotia, Canada (45°23'25.2918; – 63°15'21.1932). Soil at the Brookside experimental site as well as a climate description was provided in Chapter 3. Drainage at the site was further improved by the installation of subsurface drainage tiles at a depth of 0.8 m. A diagram of tile lines and the plot layout is found in Appendix 1.

This study was part of a larger project that was looking at year round N dynamics and losses to air and water within organic grain rotations. All field data were collected from June 1<sup>st</sup> to Sep. 10<sup>th</sup> over two years (2011 and 2012) from plots planted to spring wheat (*Triticum aestivum* L.) that followed red clover (*Trifolium pratense* L.) grown as GMr for two years. Crop rotation consisted of soybeans (year 1), two years of red clover (years 2, 3), and spring wheat (year 4) as the cash crop. The experiment was designed as a randomized block design with four main plots (fertility management treatments; 16 x 75 m) replicated over three blocks. Each main plot ( $n=12$ ) consisted of four sub-plots (crop rotation phase; 16 x 16 m) that represented all four phases of the rotation during each year of study. Each main plot (16 x 75 m) had two drainage lines placed 6.5 m apart that combined into one line at end of the plot. Between each plot was a tile-drained buffer zone (4 m) that hydrologically isolated drainage water from each plot before being diverted into an on-site heated building with tipping buckets installed below the frost line that measured drainage flow and allowed for year-round water sampling.

Treatments (i.e., FMS) differed in timing of red clover incorporation and the use of supplemental N (i.e., inorganic fertilizer and liquid dairy manure) applied prior to the spring wheat phase of the rotation. Clover was incorporated with a moldboard plow to a depth of 20 cm. Fertility Management Systems were: (1) Clover incorporated in Sep. +  $\text{NH}_4\text{NO}_3$  fertilizer applied at  $70 \text{ kg N ha}^{-1}$  prior to wheat planting (Early Fall +  $\text{N}_{70}$ ; EF+ $\text{N}_{70}$ ), (2) Clover incorporated in

Nov., but aboveground clover biomass was removed as a hay crop and liquid dairy manure applied at 70 kg total N ha<sup>-1</sup> in May prior to wheat planting (Hayed Late Fall + M<sub>70</sub>; HLF+M<sub>70</sub>), (3) Clover incorporated in Nov. (Late Fall; LF), and (4) Clover incorporated in May (Spring; SP).

In this study, we focused on in-season N dynamics during the spring wheat phase (Year 4) of the rotation and annual NO<sub>3</sub><sup>-</sup> leaching from all four phases of the rotation. The wheat variety used in this study (AC Helena) was described by Nass et al. (2001) and weed control was accomplished using standard practices for organic grain management (i.e., heavy seeding rate, 190 kg ha<sup>-1</sup>; and post-emergent weeding using a flex-tine harrow) as described by Shirtliffe and Benaragama (2014). The timing of all field activities and treatment applications is presented in Table 5.1.

**Table 5.1.** Description and chronological timing of field operations among Fertility Management Systems (FMS) during the two years of study.

| Treatments (FMS)  | Activity  | Year 1       | Year 2       |
|---|---|--------------|--------------|
| Early Fall + N <sub>70</sub><br>(EF+N <sub>70</sub> )       | Red clover incorporation                                    | 14 Sep. 2010 | 17 Sep. 2011 |
| Hayed Late Fall + M <sub>70</sub><br>(HLF+M <sub>70</sub> ) | Red clover incorporation                                    | 2 Nov. 2010  | 3 Nov. 2011  |
| Late Fall (LF)  | Red clover incorporation                                    | 2 Nov. 2010  | 3 Nov. 2011  |
| All   | N <sub>min</sub> 0–100 cm                                   | May 19 2011  | May 1 2012   |
| Spring (SP)   | Red clover incorporation                                    | 24 May 2011  | 2 May 2012   |
| Early Fall + N <sub>70</sub><br>(EF+N <sub>70</sub> )       | Fertilizer application (70 kg N ha <sup>-1</sup> )          | 8 June 2011  | 24 May 2012  |
| Hayed Late Fall + M <sub>70</sub><br>(HLF+M <sub>70</sub> ) | Liquid dairy manure application (70 kg N ha <sup>-1</sup> ) | 8 June 2011  | 24 May 2012  |
| All   | Wheat planting  | 8 June 2011  | 25 May 2012  |
| All   | Wheat plant N uptake measurement (boot stage)               | 10 Aug. 2011 | 18 July 2012 |
| All   | Wheat Harvest   | 20 Sep. 2011 | 4 Sep. 2012  |
| All   | N <sub>min</sub> 0–100 cm                                   | 2 Nov. 2011  | 10 Nov. 2012 |

#### 5.4.2 *Spring wheat yield and N uptake*

Grain yield from wheat plots was measured in Sep. of each year by plot combining a single 16 m x 1.2 m strip from the middle of each plot. Wheat uptake of N in the above ground parts of the plant was determined before head emergence (boot stage) on 10 Aug. 2011 (Year 1) and 18 July 2012 (Year 2). Aboveground biomass from five 1-m row lengths was harvested randomly from each plot ( $n=12$ ) and dried (60°C; 72 to 96 hrs). Subsamples were then ground and composited before analyses for total C and N by dry combustion using a CN analyzer (Vario Max, Elementar, Germany).

#### 5.4.3 *N<sub>2</sub>O and CO<sub>2</sub> soil emissions*

Emissions of N<sub>2</sub>O and CO<sub>2</sub> were measured in wheat plots from 1 June to 24 Aug. 2011 (Year 1) and 1 June to 17 Aug. 2012 (Year 2) with seven and six measurements in total, respectively. Measurements of CO<sub>2</sub> emissions are used in this context as a proxy for microbial activity, rather than looking at C as a GHG in a soil C budget.

A description of the chambers, method of gas sample collection, and analyses used in this study is given in Chapter 3. Cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions were calculated from June to Sep. in Year 1 and 2 (92-d) on an individual chamber basis by linear interpolation between sampling dates, using the assumption that gas flux measured on a sample date represents the daily average flux. Soil temperature (°C) and vol. water content (m<sup>3</sup> m<sup>-3</sup>) were measured (0–10 cm) in red clover (Mar.–June) and wheat plots (June and July) during gas flux measurements by recording the average of five individual readings adjacent to the soil flux chamber using standard hand held equipment. As a reminder, consideration needs to be given to the fact that all plants were removed from the inner collar area.

#### 5.4.4 *Soil properties*

Profile (0–100 cm) soil inorganic N (N<sub>min</sub>) was determined each year prior to amendment application in the spring (May) and post-harvest in the fall (Nov.) with the aid of a direct-push type, hydraulically powered soil sampler. At each sampling date, two cores were taken per subplot (i.e., rotation phase) ( $n=48$ ) for a total of 96 cores. Cores were weighed for bulk density calculations and cut into 0 to 15, 15 to 30, 30 to 60, and 60 to 100 cm sections. Surface (0–15

cm) soil was sampled by compositing 10 soil samples per plot taken with a handheld probe (2 cm i.d.). Inorganic N was extracted with 0.5 M  $\text{K}_2\text{SO}_4$  (3:1, liquid:soil) and filtered through glass fiber filter paper following the method of Keeney and Nelson (1982). Inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was analyzed colorimetrically on an auto-analyzer (Technicon Instrument Corporation, 1971).

#### 5.4.5 $\text{NO}_3^-$ leaching

Drainage water was collected from each main plot ( $n=12$ ) on a year-round basis in both Year 1 and 2. Drainage water was collected in 1-L water sampling bottles using an automated sampler (ISCO 6700 Portable Sampler, ISCO Inc., Lincoln, NE) designated for each block ( $n=3$ ). Samplers were programmed to take a 500 mL water sample from each main plot daily or every 48 h during the fall of Year 1 and 2, and less frequently (minimum weekly) as drainage slowed due to soil frost, or water deficits. Sub-samples from the 500 mL water sample were collected into scintillation vials and immediately transferred to a freezer located in the same heated building as the water samplers. During seasons of low water drainage, water samples were taken manually from the spigot on each tile drain following precipitation or thawing events. Drainage flow from each main plot area ( $1200 \text{ m}^2$ ) was measured with tipping buckets beginning in the summer of 2011 and was used to calculate annual  $\text{NO}_3^-$  losses.

Before analysis, scintillation vials were thawed over-night at room temperature before colorimetric determination of  $\text{NO}_3^-$  as described above. The concentration of  $\text{NO}_3^-$  ( $\text{mg NO}_3^- \text{-N L}^{-1}$ ) was determined on a monthly basis by taking the mean from all sample dates during that month. Cumulative  $\text{NO}_3^-$  losses ( $\text{NO}_3^- \text{-N load}$ ) were calculated by summing the product of daily tile drainage volumes ( $\text{m}^3 \text{ ha}^{-1}$ ) and concentrations that were estimated between sample dates using linear interpolation.

#### 5.4.6 Statistical analysis

Wheat N uptake, N concentration, grain yield, and cumulative emissions of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  were tested for differences in FMS ( $\alpha=0.05$ ) for each year using a mixed model with Block ( $n=3$ ) as the random factor for all plant measurements and Sub-sample ( $n=6$ ) for soil emissions. A similar analysis was used for monthly concentrations of  $\text{NO}_3^-$  ( $\text{mg NO}_3^- \text{-N L}^{-1}$ ) in drainage water and cumulative  $\text{NO}_3^-$  ( $\text{kg N ha}^{-1}$ ) losses in each year using Block as the random factor.

Emissions of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  were analyzed for the effects of FMS and the interaction with Time across all sample dates using a mixed model repeated measures analysis that treated Sub-sample as the random factor in both Year 1 and 2. A similar repeated measure analysis was used for soil  $\text{N}_{\text{min}}$  ( $\text{NH}_4^+ + \text{NO}_3^-$ ) data from the soil surface (0–15 cm), except Block was the random factor. Residual soil mineral nitrogen (RSMN) was assessed for effects on FMS, crop phase, and their interaction using a mixed model two-way analysis on soil sampled from the profile (0–100 cm) in Nov. and treating Block as the random factor. The effects of FMS, Year, and their interaction on monthly  $\text{NO}_3^-$  concentrations in drainage water were tested using a mixed repeated measures model that treated Block and Year as random factors. Soil profile  $\text{N}_{\text{min}}$  that was sampled in May from the wheat phase was evaluated for differences in FMS, Year, and their interaction using a mixed model that treated Block as the random factor. Data for  $\text{CO}_2$  and  $\text{N}_2\text{O}$  emissions, RSMN, and  $\text{N}_{\text{min}}$  from the soil surface were  $\log_{(10)}$  transformed prior to analysis to satisfy the assumptions of equal variance and normality. Following a significant FMS effect, means were separated using Tukey's mean separation test and a test of the simple effects (slices) when a significant interaction was detected. All effects were considered significant when  $p \leq 0.05$ , and statistical tests were performed using JMP software (SAS Institute Inc. Cary, NC, USA).



## 5.5 RESULTS

### 5.5.1 Precipitation, air temperature, and soil properties

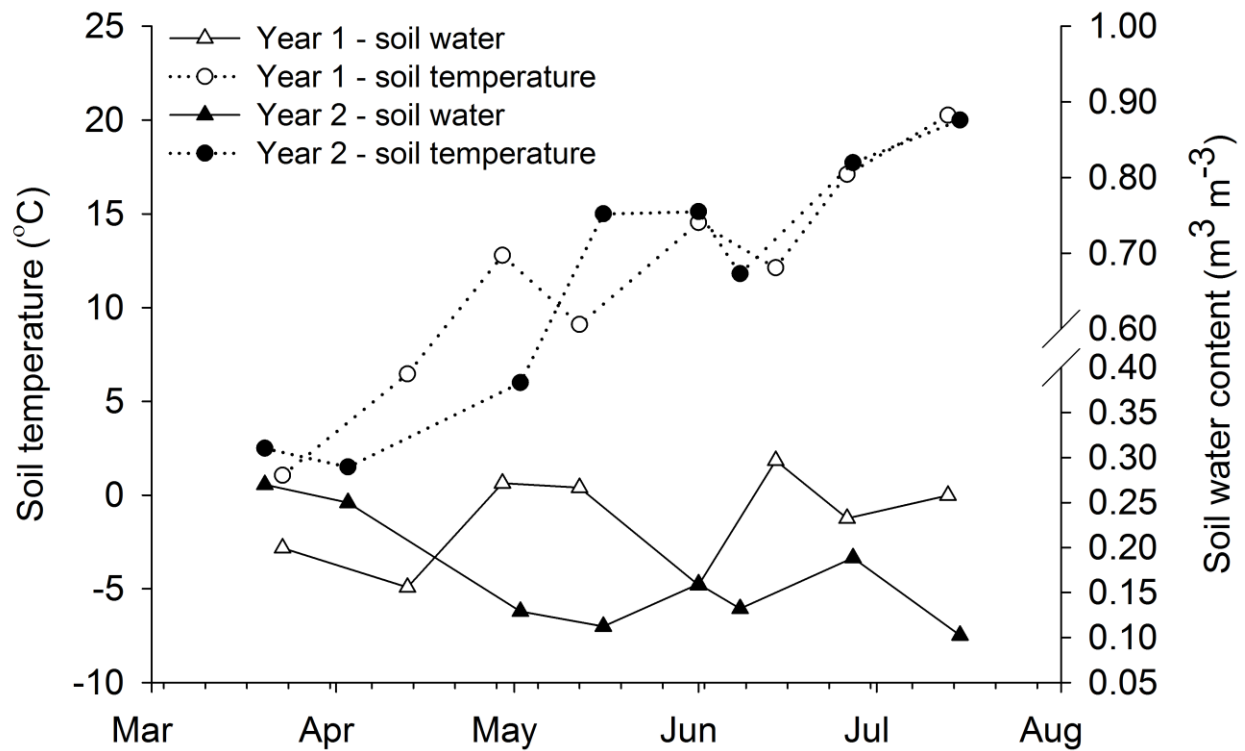
Total precipitation (rain + snow) and monthly average air temperatures from the experimental site during the two years of study are presented in Table 5.2. Overall, cumulative precipitation was slightly higher (105 mm) in Year 1 with the majority of that difference falling in the spring. Average air temperatures were not different between years, with the exception of somewhat cooler temperatures beginning in Nov. through Jan. in Year 2. Monthly average temperatures were notably similar ( $\pm 0.5^{\circ}\text{C}$ ) from May to Oct. across years, with the exception of a cooler Aug. in Year 1. However, Year 1 was characterized by a much wetter early growing season (May to July), which also delayed wheat planting (Table 5.1).

**Table 5.2.** Sum of monthly precipitation and monthly mean air temperatures as measured at the experimental site over Year 1 (2011–12) and Year 2 (2012–13).

| Month | Precipitation (mm) |        |                           | Mean air temperature ( $^{\circ}\text{C}$ ) |        |              |
|-------|--------------------|--------|---------------------------|---|--------|--------------|
|       | Year 1             | Year 2 | 30-yr (avg.) <sup>†</sup> | Year 1                                      | Year 2 | 30-yr (avg.) |
| April | 62                 | 66     | 79                        | 5.4   | 6.0    | 4.3          |
| May   | 91                 | 54     | 106                       | 11.6  | 11.6   | 10.2         |
| June  | 95                 | 62     | 96                        | 14.0  | 14.5   | 15.1         |
| July  | 119                | 55     | 91                        | 18.7  | 18.9   | 18.6         |
| Aug.  | 159                | 129    | 90                        | 18.7  | 20.1   | 18.2         |
| Sep.  | 51                 | 331    | 109                       | 15.9  | 15.6   | 13.7         |
| Oct.  | 326                | 53     | 108                       | 10.3  | 10.4   | 8.0          |
| Nov.  | 138                | 66     | 102                       | 5.5   | 3.6    | 3.0          |
| Dec.  | 128                | 101    | 74                        | 1.2   | -0.4   | -3.6         |
| Jan.  | 37                 | 17     | 54                        | -4.4  | -6.4   | -6.7         |
| Feb.  | 71                 | 45     | 44                        | -4.9  | -4.2   | -6.1         |
| Mar.  | 15                 | 108    | 59                        | 0.4   | 0.2    | -1.8         |
| Total | 1291               | 1086   | 1011                      | 7.7   | 7.5    | 6.1          |

<sup>†</sup> Climate normals (1981–2010) are from an Environment Canada weather station 20 km from the experimental site in Debert, NS.

Soil temperature ( $^{\circ}\text{C}$ ) and water content ( $\text{m}^3 \text{ m}^{-3}$ ) at the soil surface (0–10 cm) were measured from Mar. to July to estimate the potential for decomposition and N mineralization/nitrification processes (Fig. 5.1). Soil temperature increased earlier in Year 1, when compared to Year 2, which continued until June 1<sup>st</sup> when soil temperatures followed a similar pattern in both years. Soil water in Year 2 was high ( $>0.25 \text{ m}^3 \text{ m}^{-3}$ ) at the beginning of spring (Apr.), but later declined to around  $0.15 \text{ m}^3 \text{ m}^{-3}$  for the remainder of the sampling period. This was in contrast to soil water in Year 1, which began low and then remained at or above  $0.25 \text{ m}^3 \text{ m}^{-3}$  for the majority of the sampling period.



**Fig. 5.1.** Mean ( $n=12$ ) soil water ( $\text{m}^3 \text{ m}^{-3}$ ) and temperature ( $^{\circ}\text{C}$ ) from the 0–10 cm depth in plots that are transitioning from red clover to spring wheat from Mar. 15 to July 15 over two years.

### 5.5.2 Wheat Yields and N Uptake

There were no differences in wheat grain yield ( $\text{kg ha}^{-1}$ ) among FMS in Year 1 ( $p=0.68$ ) and Year 2 ( $p=0.19$ ), but annual average grain yields across all FMS were 1.6 times greater ( $p<0.0001$ ) in Year 2 ( $2683 \text{ kg ha}^{-1}$ ) as compared to Year 1 ( $1045 \text{ kg ha}^{-1}$ ). Whole plant N contents

(g N kg<sup>-1</sup>) before head emergence were significantly different ( $p= 0.03$ ) among FMS in Year 1 only (Table 5.3). The lowest tissue N concentration (g N kg<sup>-1</sup>) was obtained for the HLF+M<sub>70</sub> treatment, and the greatest for EF+N<sub>70</sub>, with both LF and SP having intermediate values of 26.2 (g N kg<sup>-1</sup>). There were no FMS treatment differences in whole plant N uptake (kg N ha<sup>-1</sup>) at head emergence in Year 1 ( $p= 0.44$ ) or Year 2 ( $p= 0.54$ ). However, when mean N uptake across all treatments was compared between years ( $p< 0.0001$ ), wheat plants had taken up 76% more N in Year 2 as compared to Year 1 and ranged from 1.2 to 1.9 kg N d<sup>-1</sup> and 2.5 to 2.8 kg N d<sup>-1</sup> in Year 1 and 2, respectively.

**Table 5.3.** Content and uptake of N by spring wheat prior to head emergence and spring wheat grain yield for Year 1 and 2 as affected by Fertility Management System (FMS). Acronyms for FMS are as follows EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring).

| FMS                 | Tissue N<br>(g N kg <sup>-1</sup> )       |               | Plant N uptake<br>(kg N ha <sup>-1</sup> ) |                  | Wheat grain<br>yield<br>(kg ha <sup>-1</sup> ) |                |
|---------------------|---|---------------|--|------------------|--|----------------|
|                     | Year 1                                    | Year 2        | Year 1                                     | Year 2           | Year 1   | Year 2         |
| EF+N <sub>70</sub>  | 30.8<br>(0.9) <sup>†</sup> a <sup>‡</sup> | 25.0<br>(3.0) | 121.63<br>(20.2)                           | 176.43<br>(14.2) | 867<br>(95.7)                                  | 2246<br>(393)  |
| HLF+M <sub>70</sub> | 25.4<br>(2.5) b                           | 24.8<br>(2.1) | 92.53<br>(25.8)                            | 158.54<br>(14.5) | 1073<br>(243)                                  | 2843<br>(222)  |
| LF                  | 26.2<br>(0.8) ab                          | 23.9<br>(1.6) | 90.22<br>(14.8)                            | 167.11<br>(15.8) | 1129<br>(52.2)                                 | 2794<br>(79.0) |
| SP                  | 26.2<br>(1.2) ab                          | 27.5<br>(1.5) | 73.68<br>(11.6)                            | 163.7<br>(3.6)   | 1110<br>(120)                                  | 2626<br>(307)  |
| <i>p-value</i>      | 0.03                                      | 0.91          | 0.44                                       | 0.54             | 0.68   | 0.19           |

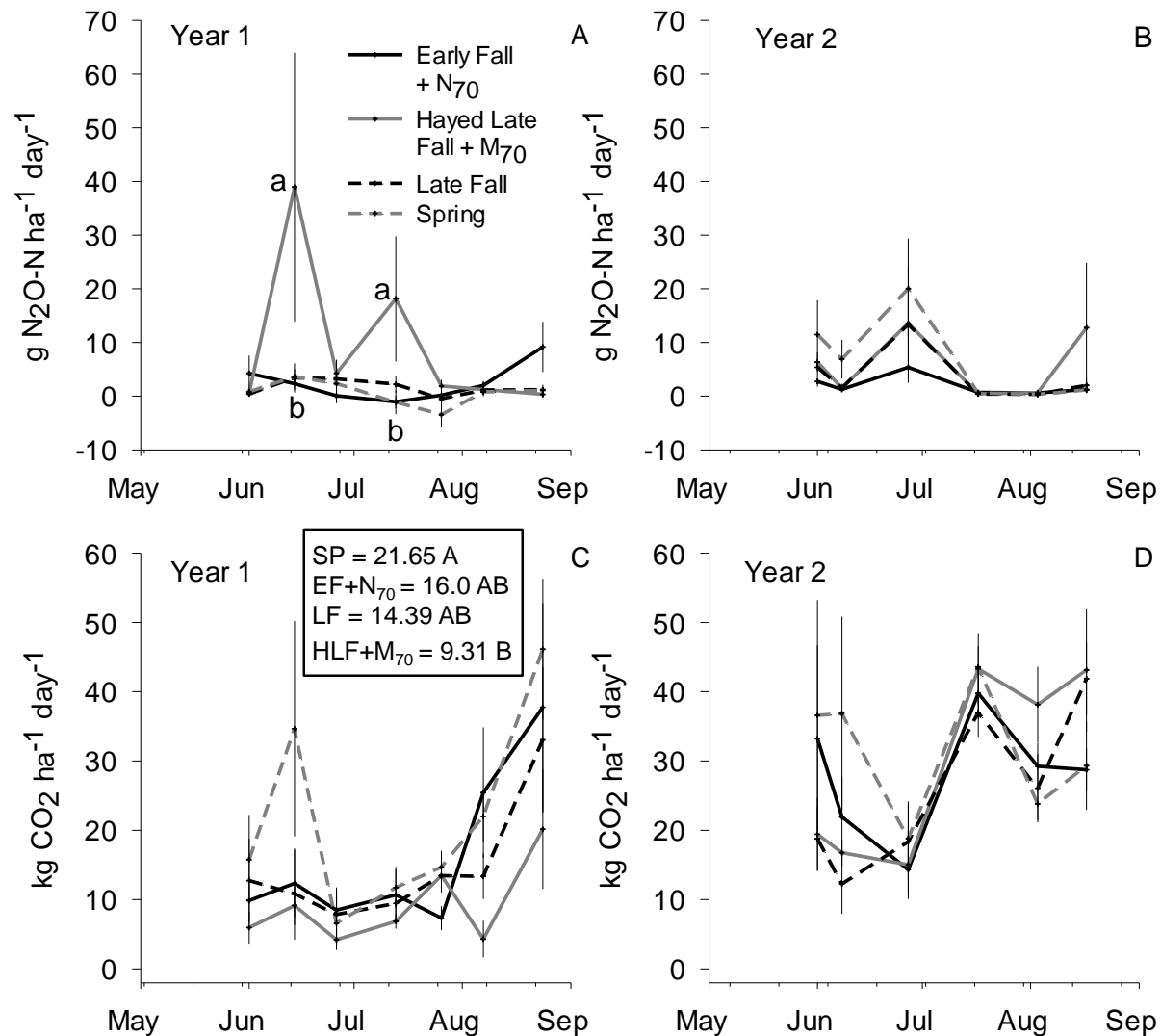
<sup>†</sup> SEM (standard error of the mean)  $n=6$

<sup>‡</sup> FMS with the same letter in a column are not different from one another ( $p > 0.05$ )

### 5.5.3 N<sub>2</sub>O and CO<sub>2</sub> emissions

Cumulative N<sub>2</sub>O emissions (kg N<sub>2</sub>O ha<sup>-1</sup>) in Year 1 (Table 5.4) were greatest ( $p= 0.04$ ) from HLF+M<sub>70</sub> plots (0.872 kg N<sub>2</sub>O-N ha<sup>-1</sup>) as compared to all other FMS that had a range of 0.057 to 0.164 kg N<sub>2</sub>O-N ha<sup>-1</sup>. In Year 2, cumulative N<sub>2</sub>O emissions were not different ( $p= 0.44$ ) among the four FMS, which had a range of 0.216 to 0.574 kg N<sub>2</sub>O-N ha<sup>-1</sup>. There was no effect of FMS in Year 1 ( $p= 0.19$ ) when N<sub>2</sub>O emissions were considered across all sample dates with a range of emissions of 0.57 to 9.34 g N ha<sup>-1</sup> d<sup>-1</sup> (Fig. 5.2 A). However, the FMS x Time interaction was significant ( $p< 0.001$ ). The greatest emission events occurred on 14 Jun. 2011 in

the HLF+M<sub>70</sub> treatment with 38.95 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> and another similar event a month later that was less than 20 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>. In Year 2, there were neither effects of FMS ( $p = 0.22$ ) on N<sub>2</sub>O emissions (Fig. 5.2 B) nor interaction with Time ( $p = 0.5$ ), but mean emission values across all sample dates had a range of 1.99 to 6.74 g N<sub>2</sub>O ha<sup>-1</sup> d<sup>-1</sup>.



**Fig. 5.2.** N<sub>2</sub>O (A, B) and CO<sub>2</sub> (C, D) emissions from the wheat phase (June–Sep.) of the crop rotation over 2 years. Dates with a significant interaction (FMS x time) in N<sub>2</sub>O and CO<sub>2</sub> daily emissions are separated for differences in fertility management systems (FMS) with different lower case letters ( $p \leq 0.05$ ). Significant effects of FMS across all sample dates are indicated in a text box with different upper case letters ( $p \leq 0.05$ ) in panel C only as effects of FMS across dates were not significant in the other analyses. Acronyms for FMS are as follows: EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring). Error bars represent the SEM ( $n=6$ ).

Across all sample dates in Year 1, CO<sub>2</sub> emissions (kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>) were highest ( $p=0.02$ ) in SP plots (21.65 kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>) as compared to HLF+M<sub>70</sub> (9.31 kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>). Intermediate values were measured from EF+N<sub>70</sub> and LF of 16.00 and 14.39 kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>, respectively (Fig. 5.2 C). There was no FMS x Time interaction ( $p=0.54$ ) in Year 1. In the second year of study (Fig. 5.2 D), there was no effect of FMS on CO<sub>2</sub> emissions (kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>) across all sample dates ( $p=0.83$ ), nor was the FMS x Time interaction significant ( $p=0.47$ ). When overall CO<sub>2</sub> emissions from all treatments and sample dates are compared, there was an increase of 87% in Year 2 as compared to Year 1. Cumulative CO<sub>2</sub> emissions (kg CO<sub>2</sub> ha<sup>-1</sup>) in Year 1 (Table 5.4) were lowest ( $p=0.0001$ ) from the HLF+M<sub>70</sub> system (874.03 kg CO<sub>2</sub> ha<sup>-1</sup>), followed by EF+N<sub>70</sub> and LF that had a mean value of 1436.81 kg CO<sub>2</sub> ha<sup>-1</sup> when compared to the SP system (2036.48 kg CO<sub>2</sub> ha<sup>-1</sup>). There were no differences ( $p=0.62$ ) among FMS for cumulative CO<sub>2</sub> emissions in Year 2.

**Table 5.4.** Cumulative CO<sub>2</sub> and N<sub>2</sub>O-N (kg ha<sup>-1</sup>) emissions among fertility management systems (FMS) from the wheat phase during 1 June to 1 Sep in each year. Acronyms for FMS are as follows: EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring).

| FMS                 | Year 1   |                    | Year 2          |                    |
|---------------------|--|--------------------|-----------------|--------------------|
|                     | CO <sub>2</sub>  | N <sub>2</sub> O-N | CO <sub>2</sub> | N <sub>2</sub> O-N |
|                     | June to Sep. cumulative emissions (kg ha <sup>-1</sup> ) |                    |                 |                    |
| EF+N <sub>70</sub>  | 1504<br>(369) <sup>†</sup> ab <sup>‡</sup>               | 0.094<br>(0.09) b  | 2564<br>(337)   | 0.216<br>(0.04)    |
| HLF+M <sub>70</sub> | 874<br>(159) b   | 0.872<br>(0.49) a  | 2878<br>(291)   | 0.574<br>(0.33)    |
| LF                  | 1369<br>(236) ab   | 0.164<br>(0.03) b  | 2545<br>(256)   | 0.359<br>(0.24)    |
| SP                  | 2036<br>(147) a  | 0.057<br>(0.08) b  | 2788<br>(437)   | 0.573<br>(0.22)    |
| <i>p</i> -value     | <b>0.01</b>  | <b>0.04</b>        | 0.86            | 0.51               |

<sup>†</sup> SEM (standard error of the mean)  $n=6$

<sup>‡</sup> FMS with the same letter in a column are not different from one another ( $p > 0.05$ )

#### 5.5.4 Soil inorganic N

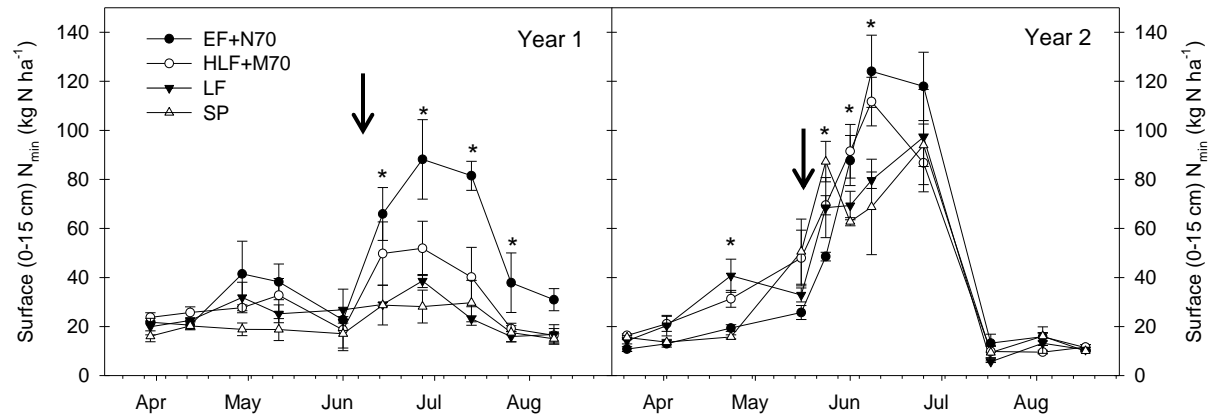
Surface (0–15 cm) N<sub>min</sub> content (kg N ha<sup>-1</sup>) measured in Year 2 clover (Apr.–June) and wheat (June–Aug.) rotation phases are presented for both study years (Fig. 5.3). When the mean for each FMS was taken across all sampling dates, an overall effect ( $p=0.047$ ) was seen in Year

1 for FMS. Mean  $N_{\min}$  content was highest for EF+N<sub>70</sub> (44.92 kg N ha<sup>-1</sup>) when compared to the lowest in SP (21.01 kg N ha<sup>-1</sup>), while the mean of LF and HLF+M<sub>70</sub> was intermediate (27.69 kg N ha<sup>-1</sup>). When differences in FMS were evaluated for each sampling date ( $p=0.029$ ), plots that received supplemental N (i.e., EF+N<sub>70</sub>, HLF+M<sub>70</sub>) were significantly greater than SP and LF through the month of June by 29 to 37 kg N ha<sup>-1</sup>. In the month of July, EF+N<sub>70</sub> was greater than all other FMS on 13 and 26 July 2011 with 81 and 38 kg N ha<sup>-1</sup>, respectively. The unamended FMS (i.e., LF and SP) resulted in consistently low (<40 kg N ha<sup>-1</sup>)  $N_{\min}$  levels when compared to FMS that were supplemented with manure and inorganic fertilizer.

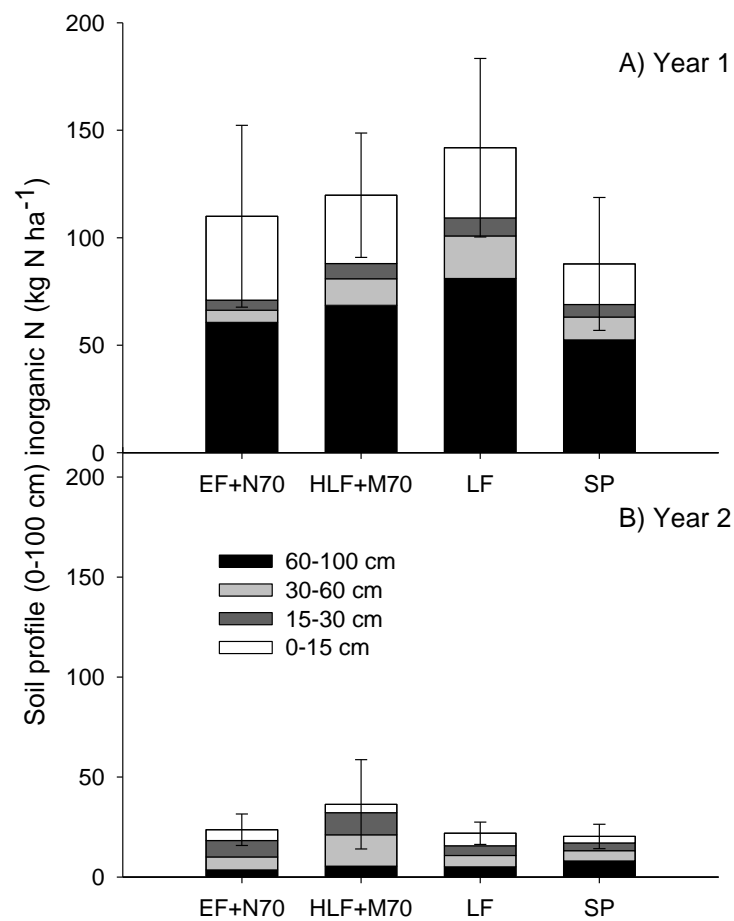
In the second year of study, there were no FMS effects ( $p=0.68$ ), but the FMS x sampling date interaction was significant ( $p<0.0001$ ). Both of the Nov. incorporated clover FMS (i.e., LF and HLF+N<sub>70</sub>) had  $N_{\min}$  contents that were double those in EF+N<sub>70</sub> and SP on 23 Apr. 2012. The smallest  $N_{\min}$  content on 24 May 2012 was from EF+N<sub>70</sub> as compared to all other FMS. Following wheat planting (i.e., 25 May 2012), the unamended FMS (i.e., EF, LF) had surface  $N_{\min}$  contents that were 36 and 59% smaller than the mean of amended FMS (i.e., EF+N<sub>70</sub> and HLF+M<sub>70</sub>) on 1 and 8 June 2012, respectively. This trend continued until the middle of July when soil  $N_{\min}$  was < 20 kg ha<sup>-1</sup> in all FMS.

Soil profile (0–100 cm)  $N_{\min}$  was evaluated from red clover plots in May, prior to SP plowdown and wheat planting, which resulted in no overall effect of FMS ( $p=0.49$ ) (Fig. 5.4), nor was the FMS x Year interaction significant ( $p=0.53$ ). However, there was a dramatic difference between years ( $p<0.0001$ ) where there was nearly four times the profile  $N_{\min}$  in Year 1, as compared to Year 2 (24.77 kg N ha<sup>-1</sup>). In Year 1, a large proportion of  $N_{\min}$  within the profile was below 60 cm (57%), while only as 27% could be found within the top 0–15 cm; this is evidence of substantial mineralization and nitrification before the sampling date, as well as the downward movement of NO<sub>3</sub><sup>-</sup> in the spring of Year 1.

There was a striking difference between  $N_{\min}$  measured at the soil surface (Fig. 5.3) and  $N_{\min}$  measured within the soil profile (Fig. 5.4) in Year 2. Approximately, 30 kg N ha<sup>-1</sup> was measured in the upper soil horizon (0–15 cm) on 23 Apr. 2012 (Fig. 5.3), yet only ~26 kg N ha<sup>-1</sup> across all FMS in the entire soil profile (0–100 cm) on 1 May 2012 (Fig. 5.4). Heavy precipitation (42 mm) during the week between sampling dates, and a difference in soil sampling methodology (i.e., composite sample vs. deep soil core) could be possible explanations for the discrepancy.



**Fig. 5.3.** Inorganic N ( $\text{NH}_4^+ + \text{NO}_3^-$ ) from the 0–15 cm soil depth for Year 1 (A) and Year 2 (B). Dates with a significant interaction (FMS  $\times$  time) are indicated with an asterisk ( $p < 0.05$ ). Acronyms for FMS are as follows EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring). Arrows indicate wheat planting. Error bars represent the SEM ( $n=3$ ).



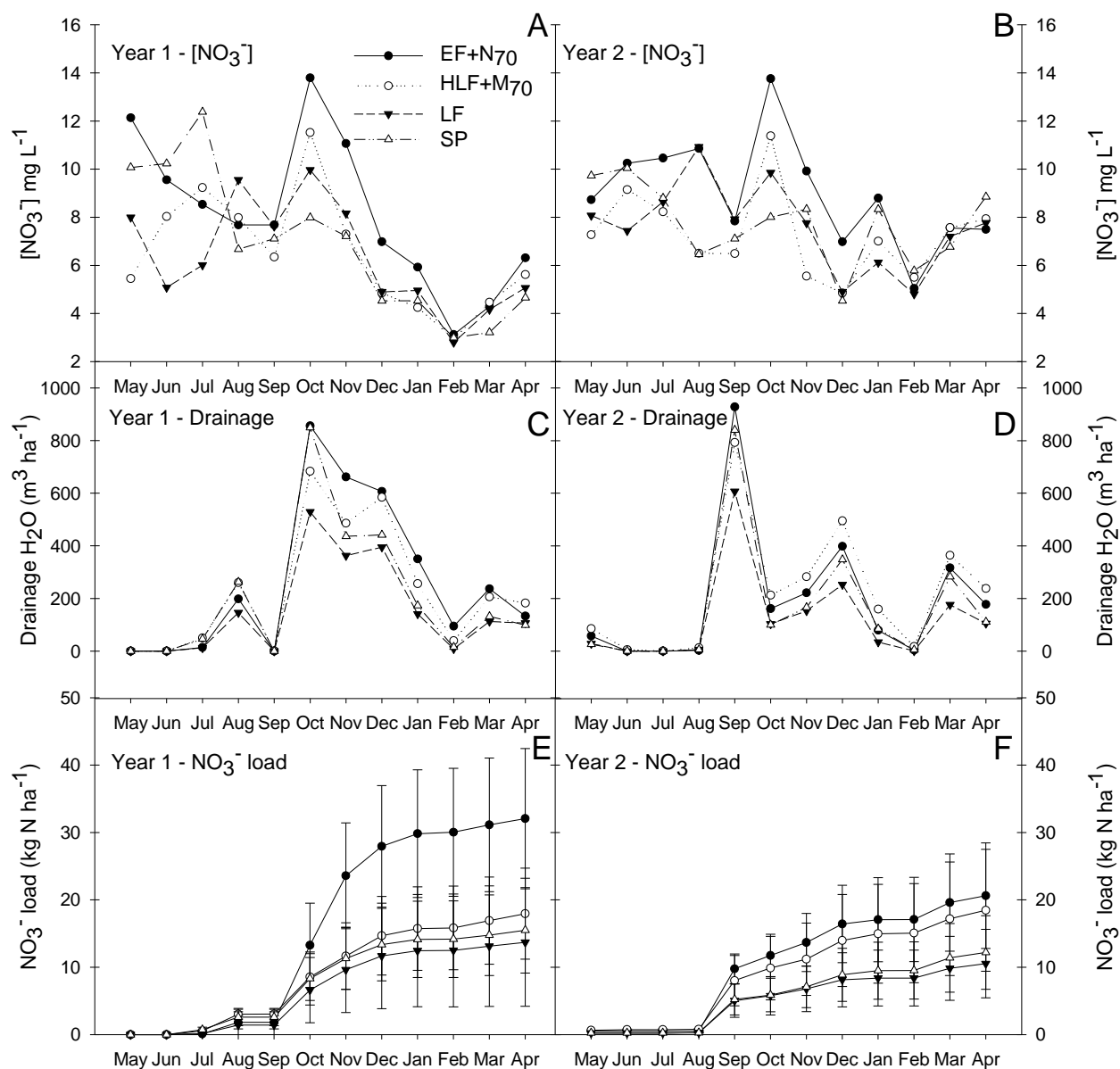
**Fig. 5.4.** Inorganic N ( $N_{\min}$ ) within the soil profile (0–100 cm) from red clover plots prior to wheat planting and sampled on (A) 19 May 2011 and (B) 1 May 2012. Categories along the x-axis refer to Early Fall + N<sub>70</sub> (EF+N<sub>70</sub>), Hayed Late fall + M<sub>70</sub> (HLF+M<sub>70</sub>), Late Fall (LF), and Spring (SP) Fertility Management Systems (FMS).

#### 5.5.5 $\text{NO}_3^-$ leaching and fall residual Soil N

Differences in monthly  $\text{NO}_3^-$  concentrations and total  $\text{NO}_3^-$  losses in drainage water among FMS were assessed using repeated measures analysis in each year of study (Fig. 5.5). There were no differences in FMS in Year 1 ( $p=0.19$ ) or Year 2 ( $p=0.09$ ), nor were there any significant interactions with Month in the first ( $p=0.59$ ), or second ( $p=0.98$ ) years of study. Similarly, cumulative annual  $\text{NO}_3^-$  losses were not significant across FMS in Year 1 ( $p=0.16$ ) or Year 2 ( $p=0.38$ ) with a range of 13.7 to 32.1 and 10.5 to 20.6 kg N ha<sup>-1</sup>, respectively.

However, when drainage water  $\text{NO}_3^-$  concentration among FMS was evaluated in a two-way analysis (i.e., FMS, Year, and their interaction) for each month there was one consistent effect (Table 5.6); EF+N<sub>70</sub> had the highest concentration of  $\text{NO}_3^-$  in drainage water in Oct., Nov., Dec., and May with values of 13.78, 10.49, 7.85, and 10.69 mg N L<sup>-1</sup>, respectively. Because drainage water is collected from the entire 4-yr crop rotation, it is appropriate to evaluate monthly mean  $\text{NO}_3^-$  concentrations over the 2-yr study, rather than direct effects of FMS in each year. Seasonal trends are similar in each year, as indicated in Fig. 5.5, but overall the highest concentrations were consistently measured from EF+N<sub>70</sub> during the months of Oct., Nov., Dec., and May. There were no significant interactions between FMS and Year during any month.





**Fig. 5.5.** Monthly NO<sub>3</sub><sup>-</sup> concentration in drainage water during Year 1 (A) and 2 (B). Drainage water volumes (m<sup>3</sup> ha<sup>-1</sup>) for each FMS in both years (C, D). Cumulative NO<sub>3</sub><sup>-</sup> losses (NO<sub>3</sub><sup>-</sup> Load) from May through Apr. (E, F). Acronyms for FMS are as follows EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring). Error bars represent the SEM (n=3).

**Table 5.5.** Concentration of  $\text{NO}_3^-$  in drainage water among Fertility Management Systems (FMS) averaged over two years Acronyms for FMS are as follows EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring).

|                            | June  | July  | Aug.  | Sep. | Oct.                 | Nov.           | Dec.         | Jan. | Feb. | Mar. | April | May          |
|----------------------------|---|-------|-------|------|----------------------|----------------|--------------|------|------|------|-------|--------------|
| <b>FMS</b>                 | ----- mg $\text{NO}_3^-$ -N L <sup>-1</sup> ----- |       |       |      |                      |                |              |      |      |      |       |              |
| EF+N <sub>70</sub>         | 9.9   | 9.5   | 9.27  | 7.76 | 13.78 a <sup>†</sup> | 10.49 a        | 7.85 a       | 7.36 | 4.08 | 5.93 | 6.91  | 10.69 a      |
| HLF+M <sub>70</sub>        | 8.59  | 8.72  | 7.25  | 6.42 | 11.45 b              | 6.42 b         | 6.45 ab      | 5.63 | 4.22 | 6.02 | 6.78  | 6.08 b       |
| LF                         | 6.26  | 7.34  | 10.24 | 7.77 | 9.91 b               | 7.96 b         | 6.1 ab       | 5.54 | 3.8  | 5.68 | 6.42  | 7.63 ab      |
| SP                         | 10.13   | 10.57 | 6.57  | 7.11 | 8.0 c                | 7.78 b         | 5.28 b       | 6.43 | 4.39 | 4.99 | 6.86  | 9.36 ab      |
| <b>Source of variation</b> | ----- Probability (p) -----                       |       |       |      |                      |                |              |      |      |      |       |              |
| FMS                        | 0.49  | 0.38  | 0.56  | 0.82 | < <b>0.001</b>       | < <b>0.001</b> | <b>0.009</b> | 0.07 | 0.81 | 0.47 | 0.96  | <b>0.001</b> |
| FMS x Year                 | 0.97  | 0.43  | 0.77  | 0.99 | 0.99                 | 0.08           | 0.49         | 0.31 | 0.94 | 0.75 | 0.61  | 0.21         |
| n =                        | 6   | 6     | 6     | 6    | 6                    | 6              | 6            | 6    | 6    | 6    | 6     | 6            |
| SE                         | 1.64  | 2.26  | 2.16  | 1.4  | 1.85                 | 1.43           | 1.15         | 1.43 | 1.25 | 1.69 | 1.82  | 0.85         |

<sup>†</sup> Values within a column with the same letter are not significantly different at the 0.05 level of probability.

Fall RSMN was determined in Nov. sampled soil cores (0–100 cm) in both years (Table 5.6). Both the FMS ( $p=0.001$ ) and the rotation phase ( $p=0.045$ ) effects were significant in Year 1, but their interaction was not ( $p=0.12$ ). Highest  $N_{\min}$  contents from the entire profile (0–100 cm) were from EF+N<sub>70</sub> and LF with 33.44 and 29.49 kg N ha<sup>-1</sup>, respectively, while HLF+M<sub>70</sub> was the smallest (20.29 kg N ha<sup>-1</sup>) and SP was intermediate with 26.14 kg N ha<sup>-1</sup>. The greatest  $N_{\min}$  content among rotation phase in Year 1 was measured from spring wheat (Y4; 29.53 kg N ha<sup>-1</sup>) as compared to the first red clover (Y2) phase (22.04 kg N ha<sup>-1</sup>), while soybean (Y1) and the second red clover (Y3) phase of the rotation were intermediate with a mean value of 28.9 kg N ha<sup>-1</sup>.

In the second year of study, the effect of FMS was marginally significant ( $p=0.054$ ), but profile  $N_{\min}$  was again highest from EF+N<sub>70</sub> (53.47 kg N ha<sup>-1</sup>) as compared to all other FMS (39.54 kg N ha<sup>-1</sup>). The effect of rotation phase was not significant ( $p=0.147$ ), but the interaction with FMS was ( $p=0.006$ ). Differences among FMS in the second red clover phase (Y3) of the rotation were significant and followed the same pattern as previously discussed for the main effects. The significant interaction was due to differences in crop phase within HLF+M<sub>70</sub>; measuring 80% more  $N_{\min}$  in the spring wheat and the first red clover phase (Y2), as compared to soybean and the second red clover year (27.86 kg N ha<sup>-1</sup>).

**Table 5.6.** Fall residual soil mineral N (RSMN) within the soil profile (0–100) cm. Soil sampled on 2 Nov. 2011 (Year 1) and 10 Nov. 2012 (Year 2). Acronyms for FMS are as follows: EF+N<sub>70</sub> (Early Fall + N<sub>70</sub>), HLF+M<sub>70</sub> (Hayed Late Fall + M<sub>70</sub>), LF (Late Fall), and SP (Spring).

| FMS                        | ----- Year 1 -----                          |                                 |                                 |                                 |   | ----- Year 2 -----             |                                |                                |                                |                                      |
|----------------------------|---|---------------------------------|---------------------------------|---------------------------------|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------------|
|                            | Soybean<br>(Y1)                             | Red<br>clover<br>(Y2)           | Red<br>clover<br>(Y3)           | Spring<br>wheat<br>(Y4)         | <b>Across<br/>rotation<br/>phase</b>        | Soybean<br>(Y1)                | Red<br>clover<br>(Y2)          | Red<br>clover<br>(Y3)          | Spring<br>wheat<br>(Y4)        | <b>Across<br/>rotation<br/>phase</b> |
|                            | <b>Crop rotation phase</b>                  |                                 |                                 |                                 |   | <b>Crop rotation phase</b>     |                                |                                |                                |                                      |
| EF+N <sub>70</sub>         | 31.93<br>(8.6) <sup>†</sup>                 | 22.8<br>(4.8)                   | 48.12<br>(9.62)                 | 30.89<br>(7.55)                 | <b>33.44</b><br><b>(9.11) A<sup>‡</sup></b> | 37.12<br>(10.03)               | 61.18<br>(21.86)               | 62.38<br>(14.39) A             | 54.21<br>(11.29)               | <b>53.47</b><br><b>(15.45) A</b>     |
| HLF+M <sub>70</sub>        | 19.6<br>(3.34)                              | 19.3<br>(1.96)                  | 17.1<br>(2.43)                  | 25.17<br>(3.15)                 | <b>20.29</b><br><b>(3.13) B</b>             | 32.13 b<br>(5.17)              | 45.96 a<br>(8.88)              | 23.58 b<br>(5.72) B            | 54.22 a<br>(13.15)             | <b>38.97</b><br><b>(10.81) B</b>     |
| LF                         | 36.7<br>(15.58)                             | 22.08<br>(4.75)                 | 27.6<br>(6.76)                  | 31.6<br>(3.38)                  | <b>29.49</b><br><b>(8.95) A</b>             | 52.41<br>(18.21)               | 35.45<br>(7.35)                | 25.82<br>(5.7) B               | 52.37<br>(8.69)                | <b>41.51</b><br><b>(12.35) B</b>     |
| SP                         | 26.02<br>(1.43)                             | 23.97<br>(5.76)                 | 24.12<br>(4.93)                 | 30.47<br>(9.04)                 | <b>26.14</b><br><b>(5.75) AB</b>            | 45.41<br>(6.18)                | 33.38<br>(7.25)                | 42.08<br>(10.79) B             | 31.66<br>(8.42)                | <b>38.13</b><br><b>(8.49) B</b>      |
| <b>Across FMS</b>          | <b>28.56</b><br><b>(9.28) a<sup>§</sup></b> | <b>22.04</b><br><b>(4.36) b</b> | <b>29.24</b><br><b>(9.11) a</b> | <b>29.53</b><br><b>(6.09) a</b> |   | <b>41.77</b><br><b>(11.36)</b> | <b>43.99</b><br><b>(13.66)</b> | <b>38.47</b><br><b>(12.99)</b> | <b>48.12</b><br><b>(11.33)</b> |                                      |
| <b>Source of variation</b> | ----- Probability (p) -----                 |                                 |                                 |                                 |   |                                |                                |                                |                                |                                      |
| FMS                        | Year 1                                      |                                 |                                 |                                 |   | Year 2                         |                                |                                |                                |                                      |
| Rotation phase (RP)        | <b>0.001</b>                                |                                 |                                 |                                 |   | <b>0.054</b>                   |                                |                                |                                |                                      |
| FMS x RP                   | 0.147                                       |                                 |                                 |                                 |   | <b>0.006</b>                   |                                |                                |                                |                                      |

<sup>†</sup> Values in brackets represent the SEM ( $n=3$ ).

<sup>‡</sup> Differences in Fertility Management Systems (FMS) are indicated in each year with different upper case letters that are significantly different at the 0.05 level of probability. Bolded upper case letters indicate main effects (across rotation phase) and plain text letters are for the FMS x RP interaction.

<sup>§</sup> Differences in Rotation Phase (RP) are indicated in each year with different lower case letters that are significantly different at the 0.05 level of probability. Bolded lower case letters indicate main effects (across FMS) and plain text letters are for the FMS x RP interaction.

## 5.6 DISCUSSION

### 5.6.1 *Soil N supply from GMr*

Although there were no differences in wheat grain yield or N uptake among FMS in either year of study, the grain yield increase in Year 2 as compared to Year 1 across all management systems suggests that the SNS was less synchronized with plant uptake in Year 1. Across all FMS and sample dates,  $N_{\min}$  (kg N ha<sup>-1</sup>) was 42% greater in Year 2 as compared to Year 1. The majority of this difference was seen during the month of June when  $N_{\min}$  was 39 and 91 kg N ha<sup>-1</sup> in Year 1 and 2, respectively. The reduction of surface soil  $N_{\min}$  to low levels (~20 kg N ha<sup>-1</sup>) also occurred earlier in the season in Year 2 as compared to Year 1, an indication of greater plant uptake in Year 2, as discussed previously. However, grain yields in Year 2 were comparable to other organic systems (Kaut et al., 2009), as well as the 50-yr average (2.6 Mg ha<sup>-1</sup>) from a fertilized fallow–wheat system in Saskatchewan, Canada (Campbell et al., 2011). Loes et al. (2011) described a 10-fold difference in barley grain yield over a 4-yr study and attributed the low yields to cold and wet soil conditions early in the growing season at two sites with similar soil texture and bulk density in Norway. Lynch et al. (2008) in previous work at the same field site as our study attributed a tuber yield reduction to a soil water deficit at the Brookside experimental site, which is an example of the influence that weather can have on the temporal variation of soil temperature and water content, and ultimately the SNS.

The bulk of the in-season SNS is derived from newly mineralized N, rather than from a carry-over effect from the fall (Zebarth et al. 2009), which makes agricultural systems that are reliant on organic N inputs from GMr vulnerable to reductions in the SNS. Wheat N uptake was at least 70% greater in Year 2 as compared to Year 1 from FMS that were reliant on organic N sources (i.e., LF, HLF+M<sub>70</sub>, SP) (Table 5.3). In contrast, N uptake in Year 2 was only 45% greater when supplemental N was applied as inorganic fertilizer and clover was incorporated in Sep. (EF+N<sub>70</sub>). The difference between FMS using organic N sources and EF+N<sub>70</sub> is further evidence that the timing for the majority of the SNS from the legume GMr in Year 1 was before

the peak N uptake time of the wheat crop. Furthermore, emissions of CO<sub>2</sub>, a measure of C availability, on 14 Jun. 2011 were highest from the SP incorporated clover system three weeks following incorporation, and then decreased to similar values measured in the other three systems (5 to 10 kg CO<sub>2</sub> ha<sup>-1</sup> d<sup>-1</sup>) 12 days later. Reduced CO<sub>2</sub> emissions from all FMS in Year 1, as compared to Year 2, is again more evidence of low heterotrophic respiration that could be explained by the observed cool and wet soil conditions in early spring of Year 1. Although there has been extensive work in recent years to improve the prediction of SNS there is still a need for regional models, as no single approach has been successful across all soils (St. Luce et al., 2011). Dessureault-Romppe et al. (2015) developed a kinetic model using a variety of soils from potato cropped fields in Atlantic Canada that were used to compare the predicted in-season SNS with actual plant uptake and RSMN with the goal of using simple soil and weather measurements to improve fertilizer recommendations in conventionally managed operations. Although the model was not developed with GMr systems in mind, there is considerable advancements being made with respect to SNS in humid climates that will likely further our understanding of the rate and timing of SNS in GMr systems. Yield reductions in GMr systems could be avoided if early season SNS dynamics were better understood by adjusting the use of supplemental N based on specific soil types, history, and weather patterns.

### 5.6.2 *NO<sub>3</sub><sup>-</sup> leaching*

Precipitation was 55% greater from Apr. through July in Year 1, as compared to Year 2 (Table 5.2), which indicates that there was more soil water in Year 1 and a larger potential for spring NO<sub>3</sub><sup>-</sup> leaching from the increased drainage. Unfortunately, we were not measuring drainage flow from Apr. to July, but NO<sub>3</sub><sup>-</sup> contents above 50 kg N ha<sup>-1</sup> below the 60 cm soil depth on 19 May 2011 (Fig. 5.4) imply that there was considerable leaching at this time. Similarly, 46 kg N ha<sup>-1</sup> were leached from red clover–spring barley rotation on a sandy loam in Sweden (Bergstrom and Kirchmann, 2004), or 71 kg N ha<sup>-1</sup> on a coarse sand in Denmark that utilized a variety of GMr rotations (Askegaard et al., 2011). The majority of NO<sub>3</sub><sup>-</sup> lost to leaching occurred during the fall, as opposed to during the growing season, which is in agreement with previous studies (Belanger et al., 2003; Lynch, 2009; Jiang et al., 2011). If the SNS is unsynchronized with crop uptake in GMr systems, there will be a large residual soil NO<sub>3</sub><sup>-</sup> pool that is susceptible to N losses. The risk for NO<sub>3</sub><sup>-</sup> leaching is even greater when the FMS uses

mineral fertilizer as a supplemental N source, as was measured from EF+N<sub>70</sub> when monthly NO<sub>3</sub><sup>-</sup> concentrations in drainage water were considered (Table 5.5). However, it is not clear if this was a result of the earliest fall clover incorporation date or the use of supplemental fertilizer in the successive wheat crop. The EF+N<sub>70</sub> FMS had the highest RSMN levels across all phases of the rotation in both years of study, in addition to having the highest RSMN from the red clover (Y3) phase of the rotation in Year 2 (Table 5.6). Considering the additional energy required to produce and transport inorganic fertilizers in addition to the apparent increase in NO<sub>3</sub><sup>-</sup> leaching losses, there is another negative aspect of the EF+N<sub>70</sub> FMS when compared to other GMr incorporation dates and supplemental N use examined in this study.

Lahti and Kuikman (2003) conducted a similar experiment that investigated multiple timings of vetch incorporation (Sep., Oct., Dec., and May) in Finland for a successive spring wheat crop. They found that soil N<sub>min</sub> increased earlier according to how early a leguminous GMr is incorporated in the fall, which poses a greater risk for NO<sub>3</sub><sup>-</sup> leaching before the cash crop phase. Askegaard et al. (2011) reported that greater NO<sub>3</sub><sup>-</sup> leaching occurred when the frequency of fall field tillage was increased in addition to earlier field tillage in the fall. We had a similar result where higher NO<sub>3</sub><sup>-</sup> concentrations were measured in tile drainage water from the EF+N<sub>70</sub> FMS in Oct., Nov., Dec., and May. A soil N supply that was unsynchronized with plant uptake, as discussed above, is also likely the cause of the increased total NO<sub>3</sub><sup>-</sup> losses in drainage water in Year 1 as compared to Year 2 (Fig. 5.5). The removal of clover biomass as hay, and the subsequent application of liquid dairy manure (HLF+M<sub>70</sub>), had no observable effect on NO<sub>3</sub><sup>-</sup> leaching when compared to the other treatments. This result was supported by no difference in surface N<sub>min</sub> and RSMN within the soil profile (0–100 cm), which was in agreement with a study conducted by Askegaard et al. (2005). Considering this, GMr incorporation in Sep. (e.g., EF+N<sub>70</sub>) is not recommended unless an adequate cover crop can be established immediately after planting, as described by Thorup-Kristensen et al. (2003) and Rasouli et al. (2014).

### 5.6.3 N<sub>2</sub>O emissions

Cumulative N<sub>2</sub>O emissions (92 d) from our study are overall quite low, with means of 0.3 and 0.43 kg N<sub>2</sub>O-N ha<sup>-1</sup> across treatments for Year 1 and 2, respectively. Emissions were comparable to an unfertilized continuous corn experiment, described by Drury et al. (2014a), with N<sub>2</sub>O emissions of 0.51 kg N ha<sup>-1</sup> from Woodslee, ON. However, emissions were much

lower than a fertilized continuous corn treatment ( $7.4 \text{ kg N ha}^{-1}$ ) also described by Drury et al. (2014a), or from a corn crop in rotation with alfalfa ( $5.8 \text{ kg N}_2\text{O-N ha}^{-1}$ ) in Rock Springs, PA (Adviento-Borbe et al., 2010). Nitrous oxide emission events were small and short lived among all FMS in Year 1 and 2, with the exception of higher emissions being measured from the HLF+M<sub>70</sub> system during two sampling dates in Year 1 following manure application. The highest daily emission occurred on 14 June 2011 in the HLF+M<sub>70</sub> FMS, six days following manure application, yet there was no simultaneous peak in CO<sub>2</sub> emissions, implying that anoxic conditions were present due to a water content of  $0.5 \text{ m}^3 \text{ m}^{-3}$  at the time of gas measurement at those specific field locations (data not shown). Water content on 14 June 2011 was on average  $0.29 \text{ m}^3 \text{ m}^{-3}$  across all 24 gas-sampling locations, as there were several days of rain prior to and during this sampling event. The localized “hot spot” phenomenon for N<sub>2</sub>O emissions was evident during this sampling event, as the influence of manure application together with the occurrence of above average water contents in these plots created conditions for increased N<sub>2</sub>O emissions. The fact that there were no N<sub>2</sub>O emission events in Year 2 following manure application on 24 May 2012 from LF+M<sub>70</sub> (Fig. 5.2) is another indication of soil conditions in Year 1 that were less than ideal for growth in the wheat crop, and the subsequent uptake of N<sub>min</sub>. The majority of N<sub>2</sub>O emissions following manure application occur within 40 d (Rochette et al., 2008; Chantigny et al., 2010) and are typically associated with high WFPS following precipitation events, in addition to the additional water contained within the slurry (Mkhabela et al., 2008). Rochette et al. (2004) measured a large N<sub>2</sub>O emission ( $830.4 \text{ g N}_2\text{O ha}^{-1} \text{ d}^{-1}$ ) three weeks following pig slurry application ( $200 \text{ kg total-N ha}^{-1}$ ) in Quebec, Canada. This emission event was attributed to denitrification due to an increase in WFPS, and the abundance of NO<sub>3</sub><sup>-</sup> and available C.

Red clover, grown in Atlantic Canada in a 2-yr rotation with fertilized ( $193 \text{ kg N ha}^{-1}$ ) potato, produced more cumulative (May to Nov.) N<sub>2</sub>O emissions ( $1.41 \text{ kg N ha}^{-1}$ ) than any other preceding crop (e.g., barley, canola, soybean); the majority of these emissions occurred immediately after potato fertilization and planting (Snowdon et al., 2013). In our study, we did not see any response in N<sub>2</sub>O emissions with fertilizer application in either year of study; however, our fertilizer application rate of  $70 \text{ kg N ha}^{-1}$  was much less than that used by Snowdon et al. (2013). Furthermore, yield-scaled N<sub>2</sub>O emissions were determined in a meta-analysis to be minimal when N surplus (i.e., applied N minus crop uptake) was in the range of 0 to  $50 \text{ kg N ha}^{-1}$  (Van Groenigen et al., 2010). Considering we had no yield or wheat N uptake response to FMS



in either year (Table 5.3), we can assume that a moderate fertilizer application of 70 kg N ha<sup>-1</sup> in addition to the incorporation of red clover the previous Sep. was not in excess of crop demand. Therefore, it is not surprising that we did not see a response in N<sub>2</sub>O emissions from this system.

#### 5.6.4 *Summary of Soil NO<sub>3</sub><sup>-</sup>, N uptake, and N losses to air and drainage water*

In temperate humid environments, such as those found in Atlantic Canada, the SNS through the growing season is from in-season N mineralization, rather than N<sub>min</sub> carried over from the previous growing season. Year to year weather variations created soil conditions in early spring of Year 1 that unexpectedly mineralized N<sub>min</sub> at a high rate and was followed by intense precipitation that distributed N<sub>min</sub> deep through the soil profile (Table 5.7). Wet soil conditions delayed wheat planting until 8 June 2011, and much of the SNS occurred too early in the growing season; as indicated by the high spring profile N<sub>min</sub> pool, and the low wheat N uptake on 10 Aug. 2011. Wheat N uptake was by far the largest N<sub>min</sub> pool in the second study year, and was 76% higher than Year 1.

Although clover removal and the application of dairy manure (i.e., HLF+M<sub>70</sub>) produced the largest emission of N<sub>2</sub>O when compared to all FMS in any year, in-season N<sub>2</sub>O emissions were low in comparison to other studies (e.g., Rochette et al., 2008; Chantigny et al., 2010; Ball et al., 2014). Furthermore, the smallest in-season N<sub>2</sub>O emissions in each year were measured from the FMS that had received a moderate application of mineral fertilizer (i.e., EF+N<sub>70</sub>), yet had the highest annual leaching losses when compared to the other FMS, which were unexpected results.

Nitrogen losses from NO<sub>3</sub><sup>-</sup> leaching are most common through the fall, but fall soil profile N<sub>min</sub> pool (RSMN; Table 5.6) was within 25.17 to 54.22 kg NO<sub>3</sub><sup>-</sup>-N ha<sup>-1</sup>, which indicated a low potential for annual NO<sub>3</sub><sup>-</sup> leaching in these GMr systems. Fall RSMN in this study was within the average range for arable soils in Quebec and Ontario from 1981 to 2006 (De Jong et al., 2009). Again, N losses as NO<sub>3</sub><sup>-</sup> in drainage tiles were low overall from all FMS, but we were unable to measure NO<sub>3</sub><sup>-</sup> leaching losses during the spring of 2011. With the majority of the N<sub>min</sub> below the rooting depth on 19 May 2011, and an additional 100 mm of precipitation over the next 42 days, there were likely large N losses as NO<sub>3</sub><sup>-</sup> in drainage water that had gone unaccounted. In environments that lack extended periods of frozen soil conditions fall plowing is typically avoided, in favor of spring or late winter GMr incorporation (Ball et al., 2007).

However, in our study there was evidence of extensive N leaching losses in spring of Year 1 and slow wheat growth, as indicated by lower CO<sub>2</sub> emissions, which likely contributed to the lowered overall wheat yields in all FMS. Surprisingly, supplemental N additions prior to wheat planting did not help alleviate the plant-available soil N deficit that occurred early in the growing season, another indication of soil conditions that were not favorable for rapid wheat growth early in the growing season of Year 1.

**Table 5.7.** Summary table of the different N<sub>min</sub> pools from fertility management systems (FMS) over two years. Spring soil profile N<sub>min</sub> was measured following fall incorporated GMr plots, but before SP incorporation. Fall soil profile N<sub>min</sub> is measured from wheat plots following harvest. Mean N<sub>min</sub> across FMS for each column is referred to as total with the SD in brackets.

| FMS                 | Spring soil<br>profile N <sub>min</sub> | Wheat N<br>uptake | In season N <sub>2</sub> O<br>emissions<br>kg N ha <sup>-1</sup> | Fall soil<br>profile N <sub>min</sub> | Annual NO <sub>3</sub> <sup>-</sup><br>leaching |
|---------------------|---|-------------------|--|---------------------------------------|---|
| ----- Year 1 -----  |   |                   |  |                                       |   |
| EF+N <sub>70</sub>  | 109.97                                  | 121.63            | 0.094  | 30.89                                 | 32.05   |
| HLF+M <sub>70</sub> | 119.76                                  | 92.53             | 0.872  | 25.17                                 | 17.94   |
| LF                  | 141.88                                  | 90.22             | 0.164  | 31.6                                  | 13.69   |
| SP                  | 87.81                                   | 73.68             | 0.057  | 30.47                                 | 15.47   |
| Mean (SD)           | 114.86 (22.4)                           | 94.52 (19.9)      | 0.297 (0.39)   | 29.53 (2.9)                           | 19.79 (8.4)                                     |
| ----- Year 2 -----  |   |                   |  |                                       |   |
| EF+N <sub>70</sub>  | 23.66                                   | 176.43            | 0.216  | 54.21                                 | 20.62   |
| HLF+M <sub>70</sub> | 36.33                                   | 158.54            | 0.574  | 54.22                                 | 18.45   |
| LF                  | 21.91                                   | 167.11            | 0.359  | 52.37                                 | 10.54   |
| SP                  | 20.28                                   | 163.7             | 0.573  | 31.66                                 | 12.18   |
| Mean (SD)           | 25.55 (7.3)                             | 166.45 (7.5)      | 0.431 (0.18)   | 48.12 (11.0)                          | 15.45 (4.8)                                     |

## 5.7 Conclusions

There was no effect of FMS on spring wheat grain yield or N uptake, but differences from year to year across all systems were significant. Lower yields and N uptake in Year 1 could be attributed to increased precipitation that encouraged the downward movement of NO<sub>3</sub><sup>-</sup> prior to cash crop planting together with wet, cool soil conditions that delayed root growth early in the growing season. Yearly differences in N uptake were reflected in the dynamics of surface soil

$N_{\min}$ , especially for FMS that were reliant on organic N sources alone. The EF+N<sub>70</sub> FMS had the highest  $\text{NO}_3^-$  concentrations in drainage water through the fall and early winter, and again during the month of May. Emissions of  $\text{N}_2\text{O}$  were small across all systems in both years, although there was a response to manure addition in Year 1 during a period of sustained precipitation and the subsequent increase in soil water content. When compared to FMS that used only GMr alone, the use of supplemental N in the spring at a moderate rate (i.e., <70 kg avail. N ha<sup>-1</sup>) did increase  $\text{N}_2\text{O}$  emissions in one year when applied as manure. Additionally,  $\text{NO}_3^-$  concentrations in drainage water increased when  $\text{NH}_4\text{NO}_3$  fertilizer was applied with EF clover incorporation, but overall N losses were minimal from all FMS. Considering the additional energy required to produce and apply supplemental N in GMr systems, it is unclear if any additional benefits to crop yields would outweigh the environmental costs of applying additional N. In this two-year study with markedly different N supplying potential early in the growing season, delaying red clover incorporation in the fall (LF) proved to have a reliable N supplying ability and a low risk to in-season  $\text{N}_2\text{O}$  emissions and  $\text{NO}_3^-$  losses to drainage tiles. These results further highlight the difficulty in linking crop uptake with soil N supply when using legume GMr, but they do provide additional evidence of the benefit of GMr in supplying N to crops such as spring wheat or other N demanding crops in eastern Canada.

## 6. SYNTHESIS AND CONCLUSIONS

Soil processes and properties that control N dynamics in agroecosystems have received by far, the greatest amount of continued interest from soil scientists in the agricultural research community during the last century. The diversity of soil N processes that occur in the soil are known to be sensitive to soil properties, climatic conditions, and crop management practices (Zebarth et al., 2003), which makes reliable predictions of N availability from organic N sources extremely difficult. The lack of long-term studies that have focused on systems that use BNF as their main N input for soil fertility has left a gap in our understanding of the agricultural N cycle and the potential for N losses as  $N_2O$  (Basche et al., 2014; Skinner et al., 2014). Furthermore, systems that include a GMr phase in their rotation often involve large amounts of N mainly derived from BNF in the year before the cash crop. Therefore, off-season losses may be of greater interest, especially in areas that experience multiple freeze and thaw cycles.

A study with four unique fertility management systems (FMS) was conducted to examine how off-season environmental N losses in the GMr phase of the rotation varied by the timing and season of GMr incorporation together with the use of supplemental N. Off-season N losses were calculated as  $N_2O$  emissions from GMr and dissolved  $N_2O$  from sub-surface drainage tiles. Following the GMr phase in the rotation was spring wheat that allowed the GMr treatments to be evaluated with respect to grain yield and in-season  $N_2O$  emissions and full-year  $NO_3^-$  losses from the tile drainage system. Treatments (i.e., FMS) differed in management by the timing of GMr incorporation and the use of supplemental N as fertilizer and dairy manure in a system that removed aboveground biomass as forage. Because each phase of the rotation was present every year, we investigated the effects of GMr incorporation and the use of supplemental N in two distinct years that began in Apr. 2011 until June 2012 (Year 1), and again in Year 2 (June 2012–June 2013).

## 6.1 Summary of Findings

Nitrous oxide emissions were overall low from all treatments in this study if you were to compare these results to some intensively managed and higher yielding production systems on finer textured soils (e.g., Gregorich et al., 2005; Drury et al., 2014a), however were comparable to annual emissions on similar coarse textured soils (Gregorich et al., 2005; Burton et al., 2008). Overall, there was a 51% increase (Table 6.1) in N<sub>2</sub>O emissions in Year 2 ( $1.37 \pm 0.13$  kg N ha<sup>-1</sup>), as compared to Year 1 ( $0.91 \pm 0.35$  kg N ha<sup>-1</sup>), but no difference in cumulative emissions among FMS ( $p>0.05$ ) in either year of study. There was a wide range of off-season (Sep. to June) N<sub>2</sub>O emissions among treatments and years from 0.26 and 1.22 kg N<sub>2</sub>O-N ha<sup>-1</sup> (Chapter 3). Overall, in-season N<sub>2</sub>O emissions were low and ranged from 0.09 to 0.87 kg N<sub>2</sub>O-N ha<sup>-1</sup> over both years of study (Chapter 5). In-season N<sub>2</sub>O emissions from the wheat crop did not differ among FMS, with the exception of an increase in N<sub>2</sub>O on two sample dates in Year 1 following manure application in the HLF+M<sub>70</sub> FMS. However, in-season emissions were only 33% of the annual emissions in both years of study (Fig. 6.1), with the greatest ( $p=0.11$ ) emissions measured from the HLF+M<sub>70</sub> system (0.87 kg N ha<sup>-1</sup>) in Year 1, and from SP ( $p=0.02$ ; 0.95 kg N ha<sup>-1</sup>) in Year 2. Furthermore, the in-season sampling period was only 25% of the days used in annual emission calculations. In comparison, 37 and 43% of the off-season emissions were measured during the month of Mar. in Year 1 and 2, respectively, with SP having the highest emissions in Year 1 (0.44 kg N ha<sup>-1</sup>) and the lowest in Year 2 (0.03 kg N ha<sup>-1</sup>).

There were no differences in cumulative N<sub>2</sub>O emissions among the three fall incorporated clover treatments in either year of study. However, the timing of N<sub>2</sub>O emissions did differ across the three fall GMr incorporated treatments, such that, greater emission events occurred within a few weeks following clover incorporation. Spring GMr incorporation did increase cumulative N<sub>2</sub>O emissions when compared to fall incorporation in the first year, yet decreased emissions in Year 2. We had a surprising pattern of N<sub>2</sub>O emissions among treatments during spring thaw that could have been connected to soil freezing in Year 2, but freezing did not explain the pattern of N<sub>2</sub>O emissions among treatments in Year 1. When freezing dynamics were similar among treatments in Year 1, the rate of thawing and the location of GMr residue likely had a greater influence on N<sub>2</sub>O emissions from the SP treatment during spring thaw. A faster drainage rate during thawing in Year 2 suggests that hydraulic conductivity was impeded in Year 1. This result

is comparable to the contrasting results from studies that examine N<sub>2</sub>O emissions from tilled and no-till systems at spring thaw (Lemke et al., 1999; Sheehy et al., 2013) and during the growing season (Ball et al., 1999; Grandy et al., 2006) that have reported differences between these systems were from an increase in WFPS and a decrease in gas diffusivity, and air-filled porosity (Rochette, 2008). It is quite possible that contrasting results among treatments in our study were influenced by soil hydraulic conductivity during spring behaving differently due to the amount of ice found within the soil profile, which would have altered the soil's air-filled porosity.

The amount of year to year variation in N<sub>2</sub>O emissions is typical of most multi-year studies, but discussions regarding variable air or soil temperatures typically remain qualitative. In this study we summed the number of temperature degree hours above and below 0°C for air and soil temperatures in an effort to quantify temporal differences in weather from year to year. The problem of relying on average daily or monthly temperature in year to year comparisons was most evident when describing weather during spring thaw. Mean air temperatures for Mar. (Table 3.4) were 0.4 and 0.2°C in Year 1 and 2, respectively, but there was actually 1034 more ΣFDH in the first two weeks of Year 1 and 806 more ΣTDH in the last two weeks of Year 1 when compared to Year 2. Daily variations in temperature during spring thaw can be substantial, and it is likely that these fluctuations will have an effect on soil processes associated with N<sub>2</sub>O emissions.

Soil N<sub>2</sub>O concentrations reached a maximum at the 15 and 30 cm depths in the SP and EF treatments on 8 Mar. 2012 with a mean (±SD) of 120±25 μL L<sup>-1</sup>, while the highest concentrations at 50 cm were measured on 11 Mar. 2012 from the EF treatment (109 μL L<sup>-1</sup>). In contrast, SP tillage had the lowest N<sub>2</sub>O concentrations at all depths in Year 2, while all other treatments had a mean of 98±20 μL L<sup>-1</sup> at 15 cm (6 Mar. 2013) and 113±37 μL L<sup>-1</sup> (13 Mar. 2013) from the 30 to 50 cm depths. Accumulated N<sub>2</sub>O from within the soil profile peaked before a significant drainage event during spring-thaw, which also increased dissolved N<sub>2</sub>O content (g N ha<sup>-1</sup>) from drainage tile water. These relationships at all soil depths suggests that soil processes throughout the profile were attributing to surface N<sub>2</sub>O emissions, rather than being solely controlled by surface conditions. Furthermore, the dynamics of N<sub>2</sub>O concentrations at soil depth proved to be a good indicator of potential N<sub>2</sub>O production and emissions from the soil surface because the highest concentrations at depth also proved to be the FMS treatments with the greatest N<sub>2</sub>O emissions (Chapters 4 and 5).

**Table 6.1.** Summary of N losses among fertility management systems (FMS) in Year 1 and 2: Surface N<sub>2</sub>O emissions, N<sub>2</sub>O dissolved in drainage water, and NO<sub>3</sub><sup>-</sup> leaching.

| Fertility<br>Management<br>Systems   | Year 1                     |                     |       |                               |   |       | Year 2                     |                  |       |                               |   |       |
|--------------------------------------|----------------------------|---------------------|-------|-------------------------------|---|-------|----------------------------|------------------|-------|-------------------------------|---|-------|
|                                      | N <sub>2</sub> O Emissions |                     |       | Dissolved<br>N <sub>2</sub> O | Leached<br>NO <sub>3</sub> <sup>-</sup> | Total | N <sub>2</sub> O Emissions |                  |       | Dissolved<br>N <sub>2</sub> O | Leached<br>NO <sub>3</sub> <sup>-</sup> | Total |
|                                      | Wheat                      | GMr                 | Total |                               |   |       | Wheat                      | GMr              | Total |                               |   |       |
|                                      | (In-<br>season)            | (Off-<br>season)    |       |                               |   |       | (In-<br>season)            | (Off-<br>season) |       |                               |   |       |
| ----- kg N ha <sup>-1</sup> -----    |                            |                     |       |                               |   |       |                            |                  |       |                               |   |       |
| Early fall +<br>N <sub>70</sub>      | 0.094                      | 0.6 ab <sup>‡</sup> | 0.694 | 0.033                         | 32.05                                   | 32.77 | 0.156 b                    | 1.221 a          | 1.377 | 0.057                         | 20.62                                   | 24.14 |
| Hayed late<br>fall + M <sub>70</sub> | 0.872                      | 0.475 b             | 1.347 | 0.04                          | 17.94                                   | 19.32 | 0.437 ab                   | 1.102 a          | 1.539 | 0.116                         | 18.45                                   | 22.63 |
| Late fall                            | 0.164                      | 0.414 b             | 0.578 | 0.021                         | 13.69                                   | 14.32 | 0.336 ab                   | 1.019 a          | 1.355 | 0.045                         | 10.54                                   | 12.92 |
| Spring                               | 0.057                      | 0.95 a              | 1.007 | 0.037                         | 15.47                                   | 16.51 | 0.949 a                    | 0.262 b          | 1.211 | 0.07                          | 12.18                                   | 14.92 |
| <i>p</i> -value                      | 0.11                       | 0.02                | 0.49  | 0.64                          | 0.23                                    | 0.25  | 0.02                       | 0.05             | 0.8   | 0.18                          | 0.32                                    | 0.32  |

<sup>†</sup> Wheat in-season refers to N<sub>2</sub>O measurements made from June to Sep. and off-season refer to measurements from Sep. to June.

<sup>‡</sup> Means within columns with the same lower case letters are not significantly different at the  $p \leq 0.05$  level of probability.

Increases in N<sub>2</sub>O emissions were linked to CO<sub>2</sub> emissions, especially following clover incorporation in both years. However, high CO<sub>2</sub> emissions in the SP treatment in Year 1 were measured prior to spring incorporation when GMr residues and new biomass remained at the soil surface. There was also a positive relationship between soil CO<sub>2</sub> and N<sub>2</sub>O concentrations at the 15- and 30-cm ( $r=0.7-0.76$ ;  $p<0.001$ ;  $n=46$ ) depths, but the strength of this relationship diminished at 50 cm ( $r=0.16$ ;  $p=0.26$ ;  $n=46$ ). Furthermore, daily surface emissions of N<sub>2</sub>O had a positive relationship with the concentration of N<sub>2</sub>O at the 30- ( $r=0.52$ ;  $p<0.001$ ;  $n=46$ ) and 50-cm ( $r=0.45$ ;  $p=0.001$ ;  $n=46$ ) soil depths throughout the winter. This relationship is substantial, as it shows that CO<sub>2</sub> production, as a result of increased microbial activity, contributed to the conditions needed for N<sub>2</sub>O production due to limited diffusion of O<sub>2</sub> from above the soil surface.

To our knowledge, concentrations of dissolved N<sub>2</sub>O measured from this study are within the range of the highest concentrations recorded in drainage water (Heincke and Kaupenjohann, 1999), and the first to make a connection of decreasing soil N<sub>2</sub>O concentrations with increasing N<sub>2</sub>O concentrations in drainage water. Overall, there was no difference in dissolved N<sub>2</sub>O ( $\mu\text{g N}_2\text{O-N L}^{-1}$ ) among treatments in Year 1 ( $p=0.7$ ) or Year 2 ( $p=0.97$ ), yet concentrations differed over time ( $p<0.001$ ). Dissolved N<sub>2</sub>O concentrations in drainage water increased during spring thaw as soil N<sub>2</sub>O concentrations decreased with maximum values in drainage water of 167 and 269  $\mu\text{g N}_2\text{O-N L}^{-1}$  on Mar. 9 and 13 of Year 1 and 2, respectively. The majority of the dissolved N<sub>2</sub>O losses occurred during a major drainage event at the peak of the spring thaw, which also corresponded to a period with the highest daily surface flux measurements. Furthermore, total dissolved N<sub>2</sub>O ( $\text{g N}_2\text{O-N ha}^{-1}$ ) were similar to the maximum flux events ( $\text{g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ ), which are known to be one of the greatest flux events of the year. Considering this, dissolved N<sub>2</sub>O losses are a significant portion of spring thaw related N<sub>2</sub>O losses in systems that have sub-surface drainage tiles.

There were no differences in wheat N uptake or grain yield among FMS in either year of study (Chapter 5), but overall there was a 76% increase ( $94.52 \pm 19.9$  to  $166 \pm 7.5 \text{ kg N ha}^{-1}$ ) in N uptake and a wheat grain yield that was 1.5 times greater in Year 2 ( $2.62 \pm 0.27 \text{ Mg ha}^{-1}$ ), as compared to Year 1 ( $1.05 \pm 0.12 \text{ Mg ha}^{-1}$ ). Lower yields and N uptake in Year 1 across all FMS could be attributed to spring soil conditions and increased precipitation that encouraged the downward movement of NO<sub>3</sub><sup>-</sup> early in the spring. The soil content of N<sub>min</sub> ( $\text{kg N ha}^{-1}$ ) at the soil surface (0–15 cm) also reflected the difference in N availability between years, as there was



nearly twice the available soil N pool in Year 2 as compared to Year 1. However, the EF+N<sub>70</sub> treatment had a greater N availability than all other FMS that were meeting N requirements through organic N sources in Year 1, indicating that GMr residue had already decomposed, and N<sub>min</sub> had moved out of the root zone. These results further emphasize the necessity of continued long-term research in systems using differing FMS so that year to year variability due to weather can be properly recognized, rather than trying to extrapolate results from a year that was influenced by a unique, or uncharacteristic precipitation event. However, the relatively large N uptake values and grain yields in Year 2 that were comparable to systems using only inorganic fertilizers provides additional evidence of the potential of GMr to supply sufficient N to crops such as spring wheat, or other N demanding crops in eastern Canada.

Unfortunately, the portion of our study that was investigating drainage volumes from our field site did not start until July of 2011, which meant we were unable to quantify all of the leaching losses that likely occurred early in Year 1. However, the fact that very high N<sub>min</sub> contents were measured below the root zone in the GMr phase of the rotation in May (Chapter 5), suggests that this N was unavailable for wheat uptake, and was likely lost to leaching. Furthermore, the trend of high N<sub>min</sub> below the root zone in spring was evident in all phases of the rotation contained within the area drained by drainage tiles (data not shown), further evidence of the rapid movement of N<sub>min</sub> below the root zone that was not just unique to the GMr phase.

The greatest NO<sub>3</sub><sup>-</sup> losses (kg N ha<sup>-1</sup>) to drainage water were measured following wheat harvest in response to high precipitation and limited plant uptake, but overall there were no difference among FMS in Year 1 (19.8 ± 8.4 kg N ha<sup>-1</sup>), or Year 2 (15.4 ± 4.8 kg N ha<sup>-1</sup>). However, the highest NO<sub>3</sub><sup>-</sup> loads (kg N ha<sup>-1</sup>) among FMS were measured in EF+N<sub>70</sub> in Year 1 (32.1 kg N ha<sup>-1</sup>) and Year 2 (20.6 kg N ha<sup>-1</sup>), which was in agreement with the overall trend of EF+N<sub>70</sub> having the highest NO<sub>3</sub><sup>-</sup> concentrations from Oct. to Jan. and again in May (Chapter 5). The amount of RSMN after harvest is known to be a good indicator of potential off-season leaching losses, which supports our findings as well. The highest RSMN across all rotation phases were measured from EF+N<sub>70</sub> plots (Table 5.6), while the phase with the soil profile N<sub>min</sub> highest among all rotation phases were the 2<sup>nd</sup> year clover plots with 48.1 and 62.4 kg N ha<sup>-1</sup> in Year 1 and 2, respectively.

In cropping systems that utilize a GMr phase in rotation with the cash crop, the season and timing of GMr incorporation, as well as the use of supplemental N, have a significant control

on the dynamics of soil N transformations and the balance of plant uptake with environmental losses. However, the greatest influence on these dynamics was from the variation in weather year to year. It is likely that improved drainage conditions via sub-surface drainage tiles contributed to the low N<sub>2</sub>O emission from the surface, but also provided a pathway for substantial dissolved N<sub>2</sub>O losses in drainage water during spring thaw.

## 6.2 Future Research

An area for future research would be the connection between GMr management and N losses to drainage water at the Brookside experimental site in Truro, NS (Appendix 1). This would complement the recent work by Drury (i.e., 2014a, and b) along with the steady stream of research from a group in Denmark (i.e., Chirinda et al., 2010b; Jabloun et al., 2015) who have been linking long-term cropping system management that involve legumes in rotation with their N losses as leached NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>O emissions from the soil surface. Because of our experimental design, our evaluation of treatment effects on NO<sub>3</sub><sup>-</sup> leaching and dissolved N<sub>2</sub>O concentrations were limited to combined effects of varied GMr management within all phases of a 4-yr rotation, rather than being able to directly quantify the effect of the GMr tillage regimes alone on concentrations of N in drainage water. Additionally, the pattern of soil freezing with respect to the timing of tillage and the incorporation of GMr biomass (Chapter 3) and the accumulation of N<sub>2</sub>O under a frozen basal layer (Chapter 4) could be further investigated in the other three phases of the rotation. Even with our estimates of dissolved N<sub>2</sub>O content, which were suggested to be conservative, the amount of N<sub>2</sub>O dissolved in drainage water that rapidly exited the drainage tiles was similar to a monthly N<sub>2</sub>O emission total from the soil surface (Chapter 3), or a large single event at spring thaw (Risk et al. 2014). Therefore, a better understanding of the seasonal and field management influenced effects of increasing N<sub>2</sub>O concentrations within the soil profile and the potential for losses as N<sub>2</sub>O dissolved in drainage water in other phases of the rotation would help us understand this rarely investigated pathway for additional N<sub>2</sub>O emissions.

The relationships between soil temperature and the magnitude of spring thaw N<sub>2</sub>O emissions need further investigation. Because snow depth has such a strong control on soil freezing behavior it is difficult to relate the accumulation of soil freezing hours (ΣFDH; Chapter 3) to the magnitude of spring thaw N<sub>2</sub>O emissions. The connection between substrate availability

and soil freezing on the disintegration of soil aggregates (Bullock et al., 1988; Dagesse et al., 2013), and microbial cell lysis or root turnover (Cleavitt et al., 2008; Elliott and Henry, 2009) with increased  $\text{N}_2\text{O}$  production does seem reasonable, but the effect of soil freezing on the hydrological controls of gas and water exchange deserves further attention.

Nitrous oxide production is intimately related to the availability of  $\text{NO}_3^-$  in the soil profile, yet the production and emission of  $\text{N}_2\text{O}$  from the soil surface and the soil content of  $\text{NO}_3^-$  are not necessarily connected. Alternatively, the availability of  $\text{NO}_3^-$  over time—as measured by the  $\text{NO}_3^-$  index (i.e., Burton et al. 2008; Snowdon et al., 2013)—is more of an integrative property that best describes  $\text{N}_2\text{O}$  emissions. A further examination of this relationship in these FMS would likely add further insight on the factors that control  $\text{N}_2\text{O}$  production. The idea of using a  $\text{NO}_3^-$  index became clear when looking at both the HLF and the LF treatment. The total and seasonal pattern of  $\text{N}_2\text{O}$  emissions was very similar among the HLF and LF treatments, although there was considerably less aboveground biomass returned to the HLF plots. When manure was applied to the HLF treatment in spring the similarities between these two treatments were not as clear when in-season  $\text{N}_2\text{O}$  emissions were considered. However, when off-season  $\text{N}_2\text{O}$  emissions are considered either the belowground contribution of substrate is more important for  $\text{N}_2\text{O}$  production, or the timing of tillage and the effects on soil pore size and distribution has a greater control. Further study on soil physical soil properties with respect to hydrological function would be warranted.

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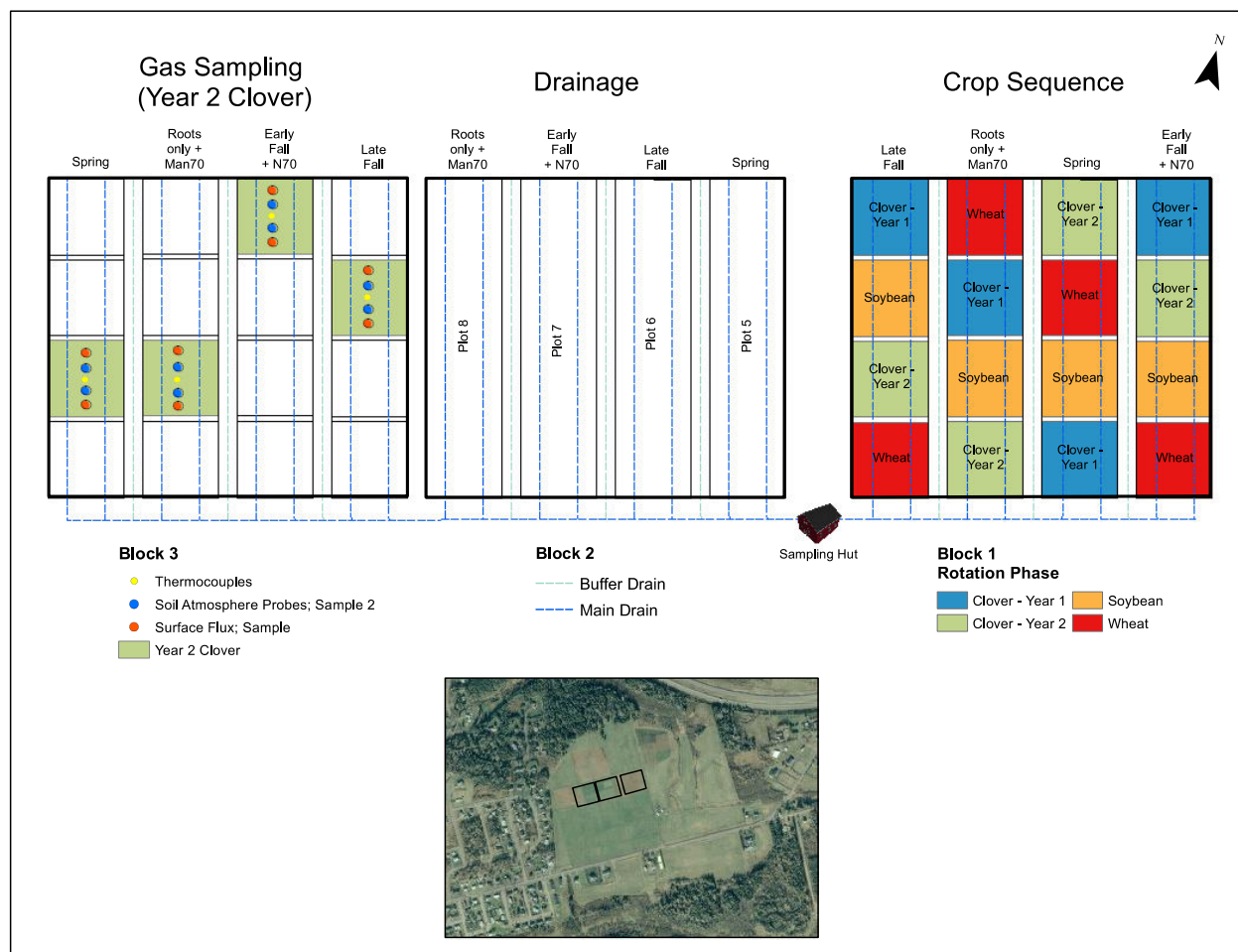
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**APPENDIX 1. SCHEMATIC OF THE BROOKSIDE EXPERIMENTAL SITE FOR ORGANIC AGRICULTURE AT DALHOUSIE UNIVERSITY'S  
AGRICULTURAL CAMPUS, TRURO, NOVA SCOTIA, CANADA.**



**Fig. 1.** Block 1 is an example of the rotation phase with four sub-plots (16 x 16 m) in each main plot ( $n=12$ ; 16 x 75 m). Spacing of sub-surface drainage tiles in each main plot is shown in Block 2 with tile lines running into the water-sampling hut. Gas sampling locations are presented in Block 3 with the location for soil temperature measurements labeled as “Thermocouple”.